IDENTIFICATION OF FACTORS ASSOCIATED WITH AND PREVENTATIVE STRATEGIES IN DIABETIC NEPHROPATHY

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Abstract

The aim of this research was to identify factors associated with and assess the efficacy of preventative strategies in the treatment of diabetic nephropathy from data collected at nephrological out-patient follow-up visits. A study of mortality, over a ten year period, in 1,421 diabetic and 26,990 non-diabetic people in Wolverhampton identified cardiovascular disease as the predominant cause of death in both groups, with female diabetic patients having an increased risk of dying from cardiovascular disease in comparison to male diabetic and female non-diabetic people. Diabetic nephropathy accounted for 4% of deaths in diabetic people. From 220 patients referred for nephrological assessment, diabetic nephropathy was diagnosed in 149. Comparison of nephropathy patients with control subjects, without nephropathy, and people with non-diabetic renal disease identified poor glycaemic control, hypertension, smoking history and defaulting from clinic visits as factors associated with nephropathy. The onset of nephropathy, retinopathy and hypertension, from diagnosis of diabetes, appeared to be accelerated in Type 2 patients, especially in Indo-Asian people.

Assessment of the efficacy of preventative treatment strategies was made by determining survival in 141 nephropathy patients over eleven years. Seven-year survival was better in a subgroup of patients with serum creatinine levels within normal limits at nephrological assessment in comparison to the main group. Five year survival of patients on renal replacement therapy was better than observed in other studies. Uncontrolled hypertension, which was

a hazard to survival, was common, and in many patients was untreated. Initial reduction of blood pressure after nephrological referral was not sustained in the majority of patients. Black patients had the worst hypertension control. The inability to speak English contributed to defaulting from routine clinic visits, was a risk for developing nephropathy and was a specific hazard for survival in Indo-Asian patients, especially in men, as was current cigarette smoking in Black patients.

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Dedication and Acknowledgements

I dedicate this thesis to all of the patients with diabetic nephropathy in Wolverhampton, without whom, I would have been unable to do this research. I also wish to acknowledge the many people who have given me help and support throughout this project; especially my supervisors, Dr. Jonathon Phillips and Dr. Cheryl Padgham, for their wisdom and endless patience, and Dr. Simon Walford whose clinical input and messages of encouragement were invaluable. I would also like to thank Dr. Paul Rylance whose enthusiasm for the topic kept me motivated, Drs. Maurice Jackson and Jonathon Odum for access to their patients' hospital records; Dr. Dev Singh for his constructive criticism; the medical secretaries and nursing staff in the diabetic and renal clinics, New Cross Hospital, who helped me to identify suitable patients; Dr. Martin Holland for providing information on the biochemical tests used at New Cross Hospital; Mr. Derek Lowe for advice on statistics and survival analysis; Mr. David Webb for helping with the computer software; Upinder Kalair, Kabir Mohammed, Claire Corston, and Moira Mottram who helped with various practical aspects of the project; Jane Biddulph for advice and encouragement; my colleagues, Julie Sinclair and Yvonne Hague for their untiring support and friendship; the Wolverhampton Kidney Patients Association for their continuing interest; and finally my family: Victoria and Alex for helping to check the computer data, Joy for nurturing me as I wrote this thesis and my eighty year old Mother for her frequent words of encouragement "if you don't hurry up and finish, I'll be dead", all of whom have tolerated my total unavailability while I wrote this thesis.

Abbreviations

AII:	Angiotensinogen II
ACE:	Angiotensin converting enzyme
ACEI:	Angiotensin converting enzyme inhibitors
AER:	Albumin excretion rate
AGEs:	Advanced glycation end-products
B:	Black
BMI:	Body mass index
Ca:	Calcium
CAPD:	Continuous ambulatory peritoneal dialysis
CHD:	Coronary heart disease
C.I.:	Confidence intervals
Con:	Control
CVA:	Cerebrovascular accident
CVD:	Cardiovascular disease
DKA:	Diabetic ketoacidosis
D.M.:	Diabetes mellitus
D.N.:	Diabetic nephropathy
DNA:	Deoxyribonucleic acid
E:	Expected
ECG:	Electrocardiogram
EDRF:	Endothelial relaxing factor
ESRF:	End stage renal failure
g:	Grams

GBM:	Glomerular basement membrane
GFR:	Glomerular filtration rate
GI:	Gastro-Intestinal
h:	Hour
H:	Hydrogen
HbA1:	Glycated haemoglobin
HD:	Haemodialysis
HDL:	High density lipoprotein
Hg:	Mercury
HG:	Hypoglycaemia
HLA:	Human leukocyte antigen
HOC:	Hyperosmolar coma
HR:	Hazard ratio
HT:	Hypertension
I-A:	Indo-Asian
ICD-09:	WHO International Classification of Disease, Ninth Revision
IDDM:	Insulin dependent diabetes
IHD:	Ischaemic heart disease
IGF:	Insulin like growth factor
kg:	Kilogram
LDL:	Low density lipoprotein
m:	Metre
MI:	Myocardial Infarction
mm:	Millimetres
µmol:	Micromolar

μM: Micromolar

n: Number

N.A.: Not applicable

N.D.: Not done

NDRD: Non-diabetic renal disease

Neph: Nephropathy

N.S.: Not significant

O: Observed

OPCS: Office of Population Censuses and Surveys

OR: Odds ratio

Out: Outliers

P: Statistical significance

PAF: Platelet activating factor

PAI-1: Plasminogen activator inhibitor 1

PDGF: Platelet derived growth factor

PROT: Proteinuria

PVD: Peripheral vascular disease

r: Correlation coefficient

R^2: Multiple correlation coefficent

RAS: Renin-angiotensin system

Ref: At referral

Rev: Revised

RET: Retinopathy

RRT: Renal replacement therapy

SD: Standard deviation

SE:	Standard error
SG:	Subgroup
TIA:	Transient ischaemic attack
t-PA:	Tissue plasminogen activator
U.K.:	United Kingdom
ULND:	Upper limit of normal diastolic pressure
ULNS:	Upper limit of normal systolic pressure
U.S.A.:	United States of America
W:	White
WG:	Whole group

Chapter 1

Introduction

1.1 Diabetes Mellitus

Diabetes mellitus is a chronic incurable disease which affects over 30 million people in Europe alone and more than 100 million world wide (Fuller, 1991; DECODE study group, 1998). There are two types of diabetes. Type1 (insulin dependent diabetes) presents in people of up to 35 years of age and requires treatment with both diet and insulin. Type 2 diabetes (non-insulin dependent diabetes) typically presents later in life but may start as early as 35 years, and can be treated with diet alone, diet and oral therapy or diet and insulin. Diabetes and its accompanying complications are major causes of morbidity and mortality throughout the western world and increasingly, in developing countries.

Type 1 diabetes is an auto-immune disease; the aetiology of which is unknown. Genetic research has shown that susceptibility to Type 1 diabetes is associated with alleles HLA-DQA1, DQB1 and DRB1 of the Human Leukocyte Antigen (HLA) region on chromosome 6 and the insulin-IGF2 region on chromosome 11 (Todd *et al*, 1987; Julier *et al*, 1991; Davies *et al*, 1994; Bain, 1995; Chauffert *et al*, 1997; Zevaco-Mattei *et al*, 1999). Although genetic susceptibility is present, diabetes does not develop until a trigger initiates the cell-mediated auto-immune response. The triggers are thought to be environmental which may include exposure to childhood viruses or toxins. The beta cells in the Islets of Langerhans in the pancreas, which normally produce insulin, are attacked and destroyed by the immune system. Subsequently, insulin secretion decreases and eventually stops (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1999).

Proinsulin, the precursor of insulin, is synthesised by the endoplasmic reticulum of the pancreatic beta cells. It is a single chain peptide which is cleaved during proteolysis to form insulin and C-peptide (Drury, 1979). Insulin is essential for the stimulation of the uptake of glucose by the brain, liver, gut and peripheral muscles; for the suppression of glucose production by the liver; and the formation and storage of glycogen in cells. It is released from beta cells when blood glucose levels rise above normal and is controlled by a negative feedback mechanism (Schmidt-Nielsen, 1983). In 1922, Banting and Macleod first used insulin to treat diabetes and the prognosis for patients with the disease changed from a few years to potentially a full life span. However, although the last surviving patient of Professor Banting's original trial of insulin died in 1993 after 71 years of successfully treated diabetes, the prognosis for many patients with Type 1 diabetes is of high morbidity and early mortality from the complications of the disease (Bliss, 1995).

Type 2 diabetes is a more complex disease in which there is insulin resistance with varying degrees of insulin deficiency. This results in the occurrence of hyperglycaemia with an over-production of insulin leading to hyperinsulinaemia in the initial stages (DeFronzo *et al*, 1992; The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1999). Although insulin is produced, it does not appear to function adequately. This may be due to abnormalities in the post prandial secretion of insulin where the initial pulse release of insulin is decreased but the subsequent consistent release is near normal. The reasons for this are not fully understood but it

may be the result of beta cell dysfunction (Leahy, 1990; DeFronzo *et al*, 1992). As blood glucose levels rise, the beta cells continue to produce insulin. Eventually, the production of insulin will stop and blood levels fall. As this happens, the level of glucose in the blood increases producing the same physiological effects of hyperglycaemia as in Type 1 diabetes. A number of studies in Type 2 diabetic patients, following oral glucose tolerance testing, have shown a deficient insulin response when compared to plasma glucose concentration, some suppression of glucose uptake by the liver and decreased suppression of hepatic glucose production (DeFronzo *et al*, 1992).

The onset of Type 2 diabetes is more insidious than Type 1 and often Type 2 patients are unaware that they are ill. The symptoms occur gradually over a period of time and can vary in severity, depending on what proportion of beta cells are still functioning. Type 2 diabetes is frequently diagnosed during routine medical examinations for work or for insurance purposes. Some patients present with the complications which are detected by doctors, opticians and chiropodists. Often patients are unaware that they have Type 2 diabetes because they do not associate the symptoms with the disease (Jackson *et al*, 1991; Singh *et al*,1992).

The complications of diabetes are the end result of badly controlled blood glucose levels often exacerbated by defaulting from routine diabetic clinic assessment (Hammersley *et al*, 1985). The complications can be divided into two categories, acute metabolic and chronic. The acute metabolic

complications can either be the result of uncontrolled high blood glucose levels e.g.: diabetic ketoacidosis or hyperosmolar coma, or secondary to excess treatment with insulin where hypoglycaemia may lead to coma.

The chronic complications can be subdivided into macrovascular, those affecting the large blood vessels, and microvascular, those affecting the small blood vessels. The macrovascular complications affect the heart, brain and the large blood vessels of the legs (Figure 1.1). Intensive insulin treatment has been shown to reduce the risk factors of cardiovascular disease in Type 1 patients (Rosenstock *et al*, 1987).

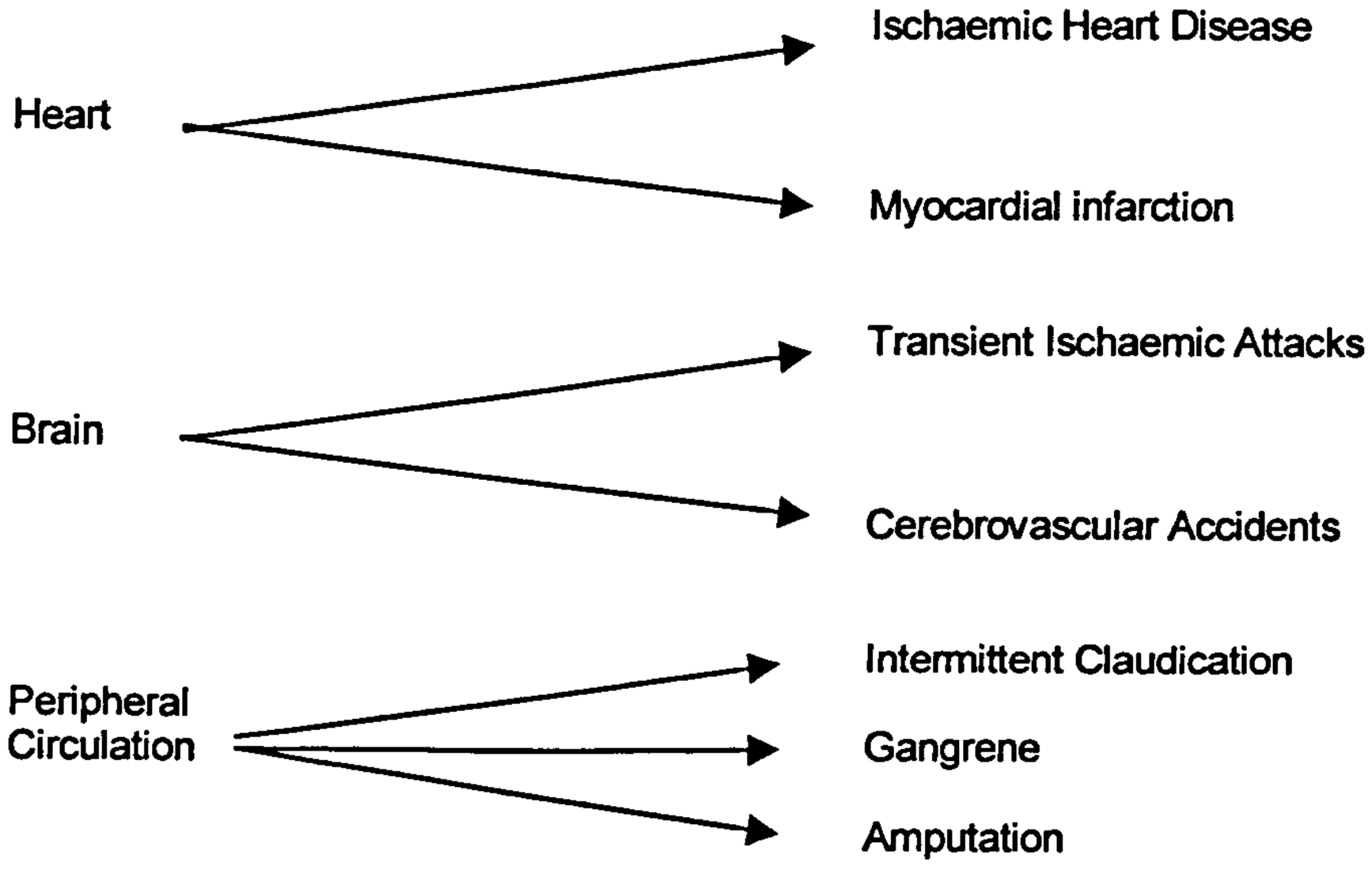


Figure 1.1: Macrovascular complications of diabetes

Patients with both types of diabetes have an increased risk of developing and dying from cardiovascular disease in comparison to non-diabetic people (Kannel and McGee, 1979; Moss *et al*, 1991; de Grauw *et al*, 1995; Gatling *et al*,1997).

The microvascular complications affect the small blood vessels in the retina, the kidney and the nervous system (Figure 1.2).

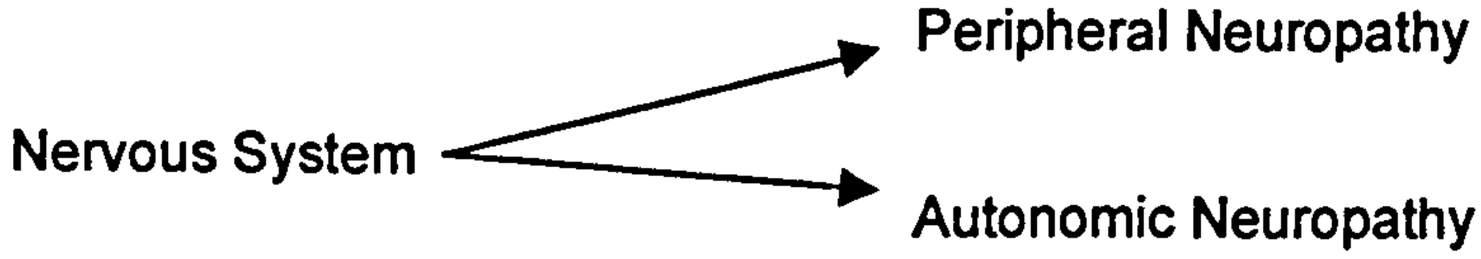


Figure 1.2: Microvascular complications of diabetes

The Diabetes Control and Complication Trial in the USA and the EURODIAB IDDM Complications study in Europe have confirmed the direct link between poor blood glucose control and the development of microvascular complications (Diabetes Control and Complications Trial Study Group, 1993; Stephenson *et al*, 1994).

The cost of diabetes and subsequent complications is high in terms of both the individual patient's quality of life and in the provision of health care for diabetic patients; in Europe alone the cost of diabetes care is thought to be 5.8% of the total expenditure on healthcare (DECODE Study Group, 1998).

1.2 Microvascular Complications

1.2.1 The Kidneys

The kidneys are paired organs located in the posterior aspect of the abdomen behind the peritoneum. The blood supply for each kidney comes from a single renal artery; occasionally there are accessory renal arteries.

The kidneys are excretory organs that remove waste products and toxins from the blood plasma during a process of filtration, reabsorption and tubular secretion. The nephron is the functional unit of the kidney. It has been estimated that in humans there are over one million nephrons per kidney (Brenner and Rector, 1986).

The nephron is composed of the Malpighian body, the proximal tubule, the loop of Henle, the distal tubule and the collecting duct. Within the Malpighian body, a small artery splits into a bundle of capillaries known as the glomerulus. Fluid is forced through the glomerular basement membrane by the blood pressure, a process known as glomerular ultrafiltration. For this to occur the blood pressure must be greater than the osmotic pressure of the blood proteins (Schmidt-Nielson, 1983; Brenner and Rector, 1986). The function of the glomerular basement membrane is to allow filtration of water and low molecular weight positively charged molecules while preventing loss of negatively charged molecules including plasma proteins, which in the normal kidney remain in the plasma after passing through the glomerular capillary network. Serum proteins of low molecular weight are filtered by the glomerulus but are then reabsorbed by the cells of the proximal tubule (Noth

et al, 1989). Following filtration, the fluid enters the proximal tubule where reabsorption of solutes, glucose and water occurs. Further reabsorption takes place in the loop of Henle, the distal tubule and the collecting ducts resulting in increasingly concentrated urine. Urine passes from the collecting ducts to the renal pelvis and is excreted via the ureters and bladder (Schmidt-Nielsen, 1983).

The capillary network within the glomerulus is lined by endothelial cells, has a central region of mesangial cells with a surrounding matrix, and finally there is a layer of epithelial cells. The central region has three layers; the *lamina rara externa*, the *lamina densa* and the *lamina rara interna* (Brenner and Rector, 1986). The glomerular basement membrane contains a glycoprotein matrix, composed of type IV collagen in the form of a triple helix with three identical chains, and carbohydrate units. The function of these carbohydrate units may be to hold the peptide chains apart and determine porosity of the membrane (Keen and Jarret, 1982). Laminin is another glycoprotein present in the glomerular basement membrane. It is composed of three sub-chains linked by disulphide bonds to form a cross shape. The majority of the membrane is composed of type IV collagen and laminin which is thought to act as a link unit between the epithelial cells, collagen and heparan sulphate proteoglycan (Tariso *et al,* 1988; Deckert *et al,* 1992). Heparan sulphate proteoglycans have been identified in the three layers of the mesangium and in the mesangial matrix and are the major anionic component which maintain the charge selectivity of the membrane, thus

preventing filtration of negatively charged plasma proteins (Deckert *et al*, 1992).

1.2.2 Aetiology of Diabetic Nephropathy

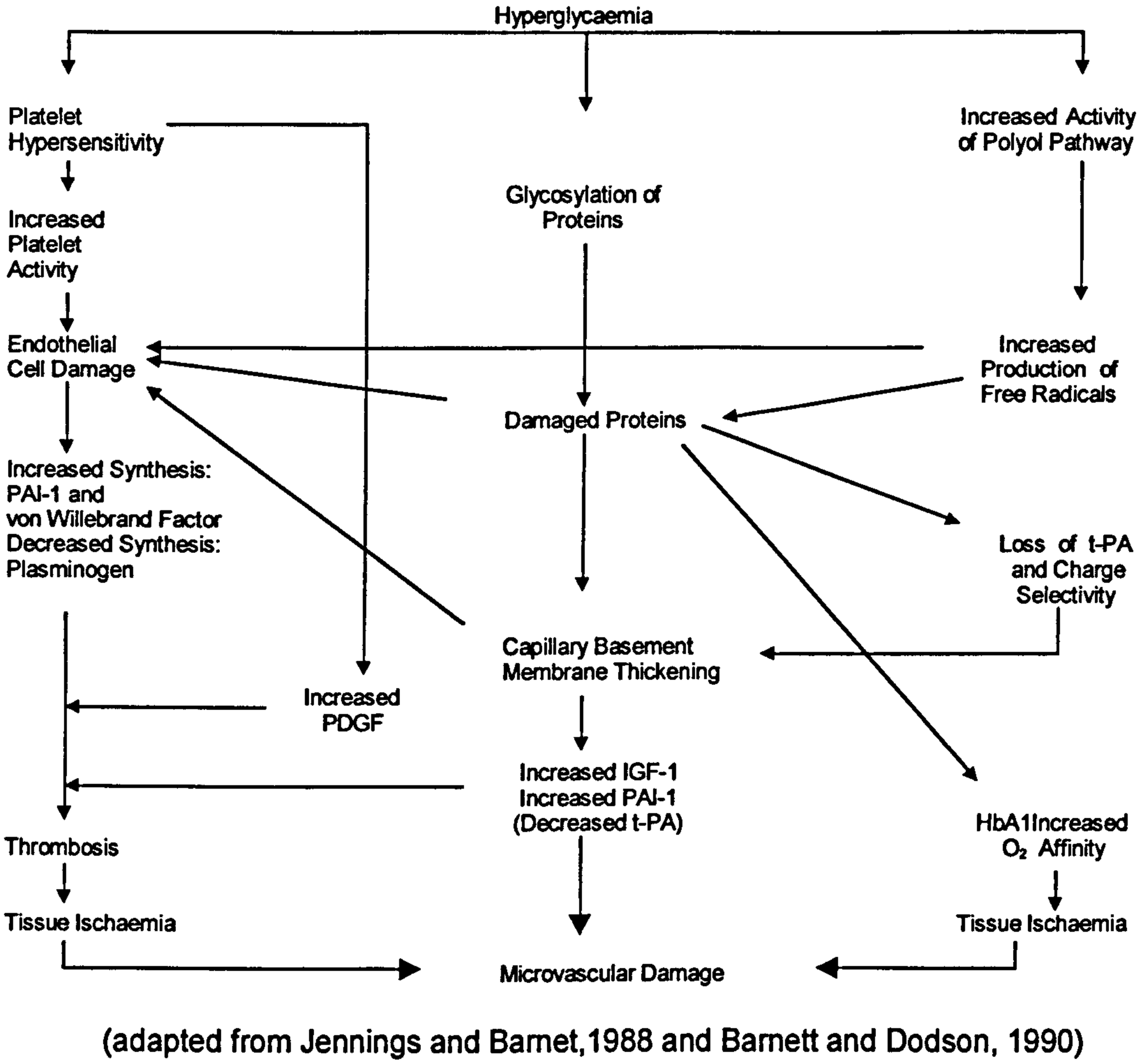
Diabetic nephropathy is the kidney disease that develops in individuals following years of poorly controlled diabetes. It can affect patients with either Type 1 or Type 2 diabetes. There appears to be a subset of patients who are particularly susceptible to developing nephropathy. It has been reported that 35% of Type 1 patients will develop nephropathy (Krolewski *et al*, 1985).

In 1989, Seaquist and co-workers demonstrated 82% clustering of nephropathy in families where one member had nephropathy in comparison to 17% in families free from the complication. Clustering of hypertension and cardiovascular disease has been shown in families of people with nephropathy (Chowdhury *et al*, 1995). It is unlikely that a single gene is responsible for the development of nephropathy. A number of potential genes have been examined in relation to nephropathy; the DD allele of the ACE gene has been implicated with decline in glomerular filtration rate in nephropathy patients (Dudley *et al*, 1995; Tarnow, 1996; Vleming *et al*, 1999). Other genes such as those encoding heparan sulphate proteoglycans, type IV collagen, insulin, aldose reductase, HLAB62 and HLADR9 genes have all been proposed as contributing to nephropathy (Tarnow, 1996; Chowdhury *et al*,1995). Although, so far, these studies are

inconclusive. A number of genetic studies are in progress with the aim of addressing this question.

1.2.2.1. *Cellular Changes in Diabetic Nephropathy*

The damage to the small blood vessels of the kidney which leads to the development of nephropathy is complex and is probably due to a combination of factors at a cellular level accompanied by haemodynamic changes. The cellular events in diabetic nephropathy involve changes to the glomerular basement membrane, alterations in coagulation and fibrinolysis plus increased activity of the polyol pathway accompanied by haemodynamic changes in the glomerulus (Figure 1.3).



(adapted from Jennings and Barnet,1988 and Barnett and Dodson, 1990)

Figure 1.3: Possible mechanisms of damage to the small blood vessels of the kidney

1.2.2.2 *The Glomerular Basement Membrane*

Increased porosity of the glomerular basement membrane and loss of plasma proteins during ultrafiltration occurs following loss of the mechanisms maintaining the negative selective charge. This is related to thickening of the membrane and loss of heparan sulphate proteoglycan. In 1989, Noth and colleagues postulated that the basement membrane contains pores of different sizes, the majority of which are small (5.1-5.7nm), that allow normal

selective filtration of solutes plus a small number of larger pores which permit unselective filtration of larger proteins.

In the presence of hyperglycaemia, blood proteins are known to undergo glycation (glycation is used instead of glycosylation to indicate that the reaction is not catalysed by an enzyme). This reaction occurs between glucose and a protein, in the absence of an enzyme, whereby the aldehyde group of the glucose molecule binds to the NH_2 of a protein forming a Schiff base which is unstable. The Schiff base rearranges to form a more stable Amadori product. In theory, both of these reactions are still reversible, but the Amadori product forms cross links with other molecules and the glycated protein formed in this irreversible reaction will remain in the body until it is naturally broken down (Cerami *et al*, 1987; Brownlee *et al*, 1988). In diabetic patients, the measurement of glycylated haemoglobin (HbA1 or HbA1c) is routinely used to assess diabetic control over the previous six weeks, as it gives an indication of mean blood glucose concentrations over this period.

The effects of glycation on the glomerular basement membrane have been investigated; when type IV collagen and laminin were incubated in high concentrations of glucose, there was increased glycation of both proteins (Tarisio *et al*, 1988). It has also been demonstrated in streptozotocin-induced diabetic rats that increase in blood glucose results in the loss of glomerular anionic sites, which represents a decrease in heparan sulphate proteoglycan in the glomerular basement membrane. However, this may or may not be the result of glycation (Moriya *et al*, 1993).

Advanced glycation end products (AGEs) and Amadori products have been found in the glomeruli of patients with nephropathy (Sakai *et al*, 1996). AGEs with modified proteins are known to stimulate mesangial cells to synthesise matrix proteins and up-regulate the production of type IV collagen (Sugiyama *et al*, 1996). The presence of glycated proteins may contribute to thickening of the glomerular basement membrane and other functional changes.

Glycation of deoxyribonucleic acid (DNA) has been demonstrated in bacterial plasmids and may occur in eukaryote cells *in vivo* (Cerami *et al*, 1987). More recently, the administration of AGEs to normal adult mice resulted in increased expression of the genes for type IV collagen and laminin (Striker and Striker, 1996). Hyperglycaemia in diabetic mice also resulted in increased expression of the messenger ribonucleic acid (mRNA) for type IV collagen and laminin but in dwarf mice carrying a mutated gene for growth hormone, there was no increase in expression of mRNA for these extracellular membrane proteins, suggesting a possible role for growth hormone in increased production of extracellular proteins and subsequent thickening of the glomerular basement membrane (Striker *et al*, 1996). It is also known that in human cell cultures, mesangial cells synthesise, secrete and bind insulin-like growth factor (IGF-1); this in turn stimulates cell production (Kojima *et al*, 1988; Aron *et al*, 1989).

1.2.2.3 *Free Radicals*

Free radicals are highly reactive cytotoxic atoms with unpaired electrons in the outer shell of their electron orbit. They are short lived because they scavenge and bind to spare electrons from a nearby atom to restore the paired electron balance; they are strong oxidants (Figure 1.4) (Jennings and Barnett,1988). Free radical production occurs in many reactions in the body including oxidative metabolism and the electron transport chain but there are mechanisms which control free radical activity. These include detoxifying enzymes (superoxide dismutase and catalase) and antioxidants (Vitamins C and E, glutathione and carotene).

$O_2 + e^- \longrightarrow O^-_2$ (Superoxide)

$O_2 + e^- + 2H^+ \longrightarrow H_2O_2$ (Hydrogen peroxide)

$H_2O_2 + e^- + H^+ \longrightarrow H_2O + OH$ (Hydroxyl radical)

$OH + e^- + H^+ \longrightarrow H_2O$

(Anonymous, From Current Debates on Diabetes, 1992)

Figure 1.4: Free radical production

The sorbitol pathway is affected by hyperglycaemia in that high glucose levels result in increased use of NADPH and sorbitol accumulation (Section 1.2.2.4). This use of NADPH eventually results in reduced synthesis of antioxidants (Barnett and Dobson, 1990). People with diabetes are known to have decreased levels of the antioxidants glutathione and Vitamin C. Oxidation of lipoprotein by free radicals produce lipid peroxides. These stimulate platelet aggregation and fibrin formation while decreasing

prostaglandin synthesis by endothelial cells. Hyperglycaemia is thought to promote lipid peroxidation, thereby increasing the risk of vascular damage.

Non-enzymatic glycation alters the activity of enzymes, affects co-enzymes and ultimately leads to the production of free radicals. Glycation of superoxide dismutase results in an Amadori product which generates superoxide (O^-_2) and hydrogen peroxide (H_2O_2) which in turn produces the hydroxyl radical (OH) (Fujii *et al*, 1996). Extracellular superoxide is transported into the cell which causes an increase in intracellular calcium ions which in turn activates synthesis of nitric oxide (Ido *et al*, 1996). Combination of superoxide and nitric oxide produces the reactive peroxynitrite ion, this decomposes to form hydroxyl free radicals (Bruckdorfer, 1993). Increased nitric oxide synthesis by endothelial cells results in vasodilation and vascular permeability and production of hydroxyl free radicals leads to oxidative damage to cells (Tomlinson, 1994; Ido *et al*, 1996)

1.2.2.4 *The Polyol (Sorbitol) Pathway*

It has been suggested that the polyol pathway is intimately involved in the mechanisms which cause microvascular disease in the kidney, eyes and the nerves. In the presence of hyperglycaemia, there is increased production of aldose reductase, possibly as a means of regulating osmolarity in the extracellular fluid. Aldose reductase catalyses the reaction where D-glucose

is converted to sorbitol in the presence of NADPH (Tomlinson, 1994) (Figure 1.5).

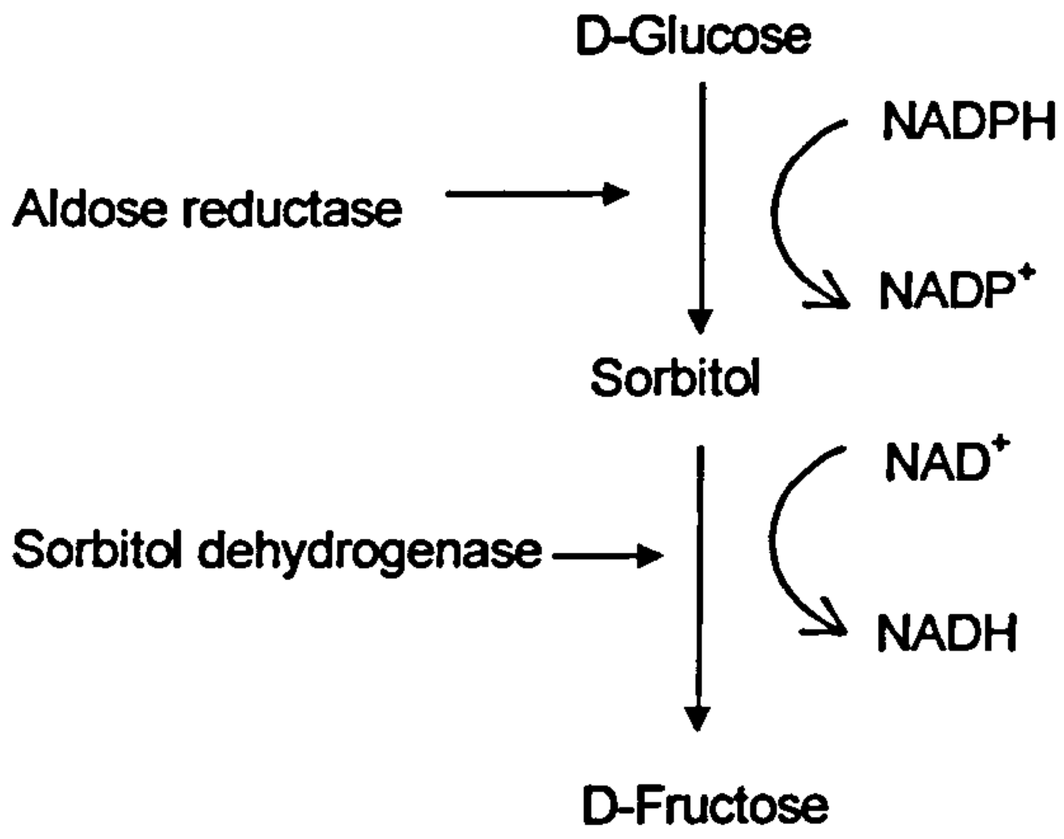


Figure 1.5: Sorbitol pathway (Tomlinson, 1994)

The consumption of NADPH during this reaction by aldose reductase is high. NADPH is needed to produce antioxidants, therefore their production is decreased during hyperglycaemia leaving tissues susceptible to damage by free radicals.

1.2.2.5 *Alterations to Blood Coagulation and Fibrinolysis*

It has been suggested that thickening of the glomerular basement membrane is a response to capillary endothelial damage or raised capillary pressure (Tooke, 1987). However, other factors affecting endothelial cells may contribute to the changes in nephropathy. Interleukin-1 has an inhibitory effect on the synthesis by endothelial cells of tissue plasminogen activator (t-PA), an essential component of fibrinolysis - the breakdown of fibrin in blood clots (Gough and Grant, 1991).

Endothelial cells have a number of important functions in regulating haemostasis including:

(a) synthesis of:

- plasminogen (the substrate of the fibrolytic system)
- von Willebrand factor (promotes platelet adhesion)
- platelet activating factor (PAF) (stimulates aggregation of platelets)
- prostacyclin (an inhibitor of platelet aggregation)

(b) contributing to thrombus formation by binding fibrinogen

(c) producing thrombomodulin (which binds thrombin while simultaneously binding heparan sulphate, an essential co-factor for anti-thrombin III and is also important in the protein C anticoagulant system)

(d) producing endothelial relaxing factor (EDRF) (which controls local vascular tone).

Abnormalities of production of EDRF may contribute to the haemodynamics found in nephropathy (Tooke, 1987; Remuzzi and Rossi, 1989). Thus damage to the endothelial layer of the glomerulus produces abnormalities of haemostasis which may in turn contribute to further renal pathology.

In the presence of hyperglycaemia, platelets demonstrate increased adhesiveness and aggregation (Jones and Peterson, 1981). In 1979, Waitzman proposed that there is disruption to the production of prostaglandins, including prostacyclin, in diabetic patients with microvascular disease. It is postulated that this disruption alters the mechanisms which normally prevent platelet aggregation. Increased activity of platelets may damage endothelial cells thus stimulating abnormal production of platelet

activating factor (PAF) and plasminogen activator inhibitor 1 (PAI-1) leading to the release of platelet derived growth factor (PDGF) and Interleukin-1.

A recent study comparing Type 2 diabetic patients with non diabetic controls demonstrated that platelet aggregation was the same for both groups (Jokl et al, 1995). However, in the diabetic patients platelets released significantly more PAI-1, which correlated with low levels of very low density lipoprotein (VLDL) cholesterol and high plasma triglyceride levels (Jokl *et al*, 1995). A number of studies of diabetic patients with reduced renal function, varying from microalbuminuria to overt nephropathy, have shown increased concentrations of lipids and plasma fibrinogen and enhanced platelet activity and aggregation (Rylance *et al*, 1986; Jensen *et al*, 1988; Jones *et al*, 1989 and O'Donnell *et al*, 1991: Omoto *et al*, 1999).

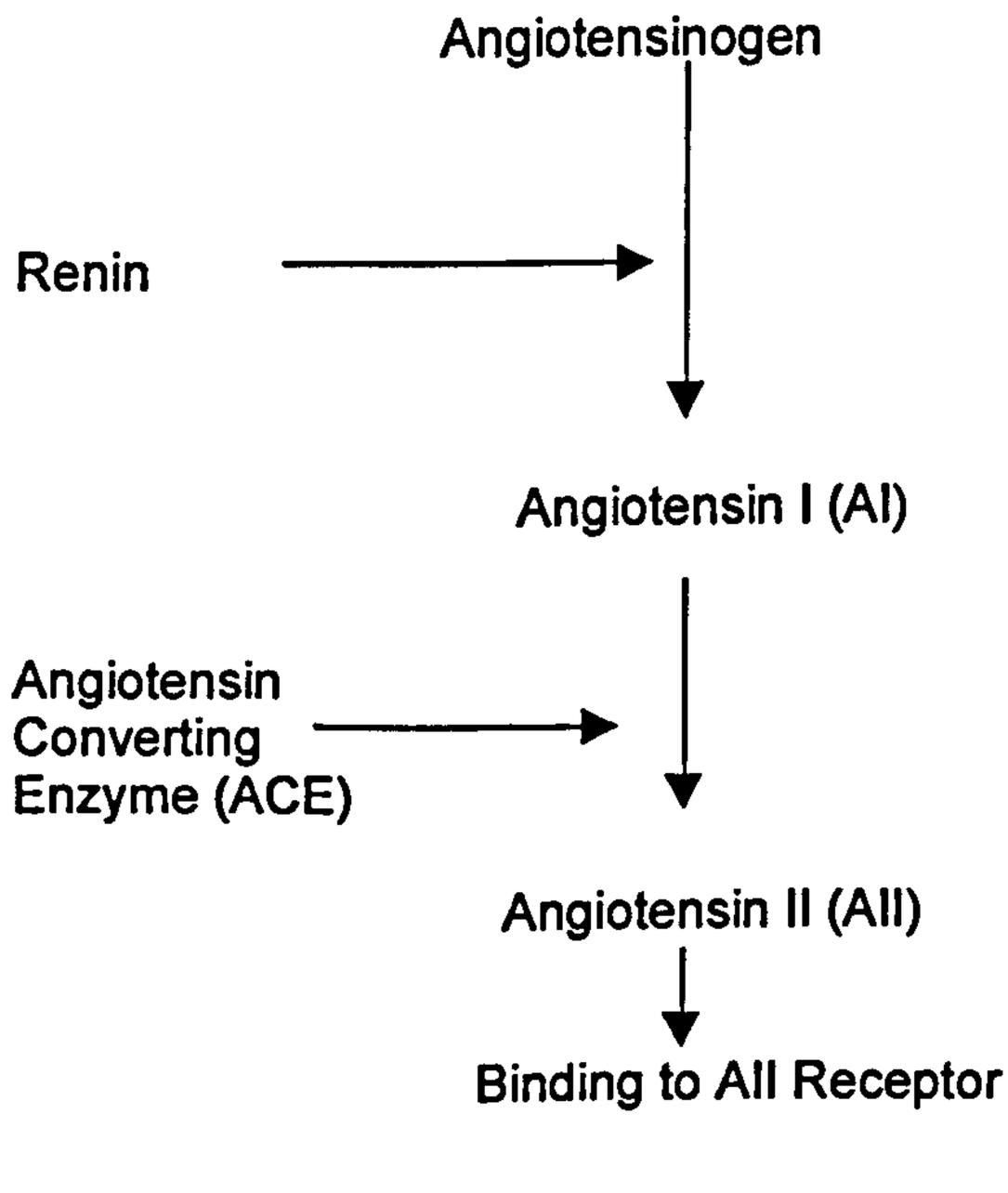
1.2.2.6 Lipids in Diabetic Nephropathy

Patients with well controlled Type 1 diabetes have similar lipid profiles to non-diabetic people, whereas those with poorly controlled diabetes have elevated lipid levels (Betteridge, 1989). In contrast, Type 2 patients tend to have low HDL cholesterol and raised total and very low density lipoprotein triglyceride levels (Betteridge, 1989). Hyperlipidaemia has been demonstrated to be linked with hyperinsulinaemia and may be an integral part of the development of Type 2 diabetes (Frayn, 1993).

It has been demonstrated that in both types of diabetes, patients with microalbuminuria and those with proteinuria have raised levels of total cholesterol, low density lipoprotein (LDL) cholesterol and triglyceride (Eckel *et al*, 1981; Watts *et al*, 1988; Seghieri *et al*, 1990).

1.2.2.7 The Role of the Renin–Angiotensin System in Diabetic Nephropathy

Much research had been undertaken looking at the role of the renin-angiotensin system (RAS) in diabetic nephropathy (Figure 1.6).



(adapted from Barnett and Dobson, 1990)

Figure 1.6: Renin-Angiotensin System

The RAS works by a negative feedback mechanism in the kidney and is designed to maintain sodium balance and circulating blood volume (Padfield,

1988; Davidson *et al*, 1998). It is activated by reduced blood pressure, decreased blood volume, Na^+ depletion and sympathetic stress. Renin, a proteolytic enzyme, is released in response to the stimulants previously mentioned, and acts on angiotensinogen which is converted to angiotensin I (AI). This is turn is converted to angiotensin II (AII) by the action of angiotensin converting enzyme (ACE). The effects of AII binding to specific AII receptors are to produce systemic vasoconstriction and stimulate the following:

- aldosterone synthesis and secretion (by the adrenal cortex) which promotes sodium reabsorption by the proximal tubule of the kidney
- hypophyseal vasopressin secretion
- inhibit the release of renin in the kidneys.

The RAS is inactivated when these conditions return to normal. The synthesis of RAS components occurs in a variety of cells relevant to nephropathy: renin and angiotensinogen are synthesised in endothelial, vascular smooth muscle cells and the liver; ACE is produced in vascular endothelial cells and AII in the vasculature. AII receptors are found in several organs including the vasculature smooth muscle, endothelial cells, the kidney and the heart (Osbakken, 1995).

Diabetic patients, even with moderately good diabetic control, have an increased blood volume. This is due to the co-transport of sodium ions to the extracellular fluid which occurs with reabsorption of glucose by the renal tubules, leading to dilatation of the small blood vessels (Anderson and Brenner, 1988). This in turn causes adaptive hyperfiltration and

hyperperfusion in the micro-circulation of the glomerulus. The specific affect of AII in the kidney is to increase the glomerular capillary pressure. Glomerular hyperfiltration is found in the early stages of diabetic nephropathy and is caused by a combination of elevated glomerular plasma flow and increased glomerular capillary hydraulic pressure (Zatz *et al*, 1986).

1.3 Clinical Aspects of Diabetic Nephropathy

1.3.1 Clinical Presentation of Diabetic Nephropathy

Five stages have been described in the development of diabetic nephropathy in Type 1 diabetic patients (Selby *et al*, 1990). The first stage occurs within the first five years following diagnosis of diabetes. There is hyperfunction of the kidney demonstrated by an increased glomerular filtration rate (GFR). On microscopic examination of renal biopsy specimens, the structure of the kidney appears to be normal, the albumin excretion rate (AER) and the blood pressure are both normal. The link between kidney function and blood pressure control is intimate; within the kidney are the components of several biochemical pathways including the RAS whose reactions influence vasoconstriction and vasodilation of blood vessels and ultimately the level of arterial blood pressure (Castiglioni and Savazzi, 1988; Selby *et al*, 1990).

As the disease progresses to stage two, the glomerular structure alters and the glomerular basement membrane starts to thicken. The GFR may remain raised or return to normal while the AER and blood pressure remain normal. By stage three, the loss of small amounts of protein in the urine, known as microalbuminuria, becomes detectable. Microalbuminuria is defined as AER of 20-200 μg of protein $minute^{-1}$ or 30-300 mg $24hr^{-1}$ (Castiglioni and Savazzi, 1988; Deckert *et al*, 1991). The GFR may still be raised, normal or starting to decline as the kidney function deteriorates while the blood

pressure will be near the upper levels of normal or slightly raised. It was once thought that microalbuminuria rarely occurred in Type 1 patients before five years duration of diabetes (Castiglioni and Savazzi, 1988). However, recently the results of two studies, the EURODIAB IDDM Complications study and the WHO Multinational Study of Vascular Disease in Diabetes, have demonstrated that 19% (EURODIAB Study) and 15% (WHO study) of Type 1 patients had microalbuminuria within five years of diagnosis of diabetes (Stephenson et al, 1994).

The progression to stage four of the disease is marked by the excretion of increasing amounts of protein in the urine until frank persistent proteinuria occurs, detectable on dipstick testing. At this point the AER is greater than 200 μg protein minute^{-1} or greater than 300mg of protein excreted every 24 hours. The blood pressure will now have risen to hypertensive levels and require therapy. Proteinuria is thought to reach a peak after sixteen years duration of diabetes in susceptible Type 1 patients (Viberti, 1988).

Stage five is the final progression to end stage renal failure (ESRF), by which time the GFR will have declined to less than 10 ml minute^{-1}. The AER may either be high, indicating continuing renal damage, or low as a result of loss of kidney function. Without renal replacement therapy (RRT), death would rapidly occur.

In the past the development of microalbuminuria in Type 2 diabetes was regarded as a sign of incipient cardiovascular disease as often the diagnosis

of ischaemic heart disease or death from myocardial infarction frequently occurred within a relatively short time of detection of microalbuminuria (Mogensen, 1984; Viberti, 1988). It is possible that a more aggressive approach to the treatment of both Type 2 diabetes and ischaemic heart disease has resulted in patients surviving for longer after microalbuminuria is detected allowing renal disease to progress to proteinuria. The diagnosis of diabetic nephropathy in Type 2 diabetes is less easily diagnosed in that older patients may have other types of renal disease, including renal artery stenosis, accompanying nephropathy. However, there are contradictory reports on this topic and even histological evidence is not always definitive (Waldherr *et al*, 1992; Ritz *et al*, 1995). In Wolverhampton, a diagnosis of diabetic nephropathy is made on clinical grounds based on a history of diabetes, the presence of diabetic retinopathy and hypertension which has developed around the same time as proteinuria. This excludes renal disease secondary to hypertensive changes in the kidney.

Type 2 patients may present at diagnosis of diabetes with proteinuria, due to nephropathy, accompanied by diabetic retinopathy and other complications (Turner *et al*, 1996). Many Type 2 patients are unaware of the symptoms of diabetes which, if untreated, can result in hyperglycaemia over a considerable period of time which in turn leads to microvascular damage and the resultant complications (Jackson *et al*, 1991; Singh *et al*, 1992). It is probable that the early disease process of nephropathy in Type 2 patients follows the same course as in Type 1 patients. It has been reported that in Type 2 patients the time to onset of proteinuria is shorter and that the

progression of renal disease is slower than in people with Type 1 diabetes (Ritz and Stefanski, 1996; Christensen *et al*, 1999).

1.3.2 Treatment and Prevention of Diabetic Nephropathy

1.3.2.1 Glycaemic Control

An early review article on the relationship between poor glycaemic control and microvascular complications concluded that there were no definitive results from studies in humans which demonstrated this relationship, although animal studies had strongly suggested a link between the two (Tchobroutsky, 1978). However, in 1987, Nyberg and co-workers demonstrated that glycaemic control was related to GFR in patients with diabetic nephropathy. The use of intensified insulin therapy and subcutaneous insulin infusion has resulted in retardation of established microvascular complications and prevention of the development of these in Type 1 patients as has the use of intensive oral therapy in Type 2 patients (Feldt-Rasmussen *et al*, 1986; Reichard *et al*, 1993; Diabetes Control and Complications Trial Research Group, 1993; American Diabetes Association, 1999). As a result, improvement in glycaemic control is regarded as the most important preventative measure in relation to all microvascular complications.

1.3.2.2 Anti-Hypertensive Therapy

The use of angiotensin converting enzyme inhibitors (ACEI) in the treatment of early diabetic nephropathy in animals and humans has shown that alterations in renal haemodynamics occur even when the drugs do not affect the systemic blood pressure (Zatz *et al*, 1986; Marre *et al*, 1991). Reddi and colleagues have demonstrated that treatment of diabetic rats with an ACEI, enalapril, improved albumin excretion rate by preventing loss of heparan sulphate proteoglycan. From this it can be concluded that ACE inhibition restored the negative selective charge of the glomerular basement membrane (Reddi *et al*, 1991).

Two early studies demonstrated irrevocably that controlling hypertension in patients with nephropathy, using different types of anti-hypertensive medication, had a beneficial effect by preventing a reduction in GFR and by reducing albumin excretion rate and arterial blood pressure (Mogensen, 1982; Parving *et al*, 1983). The introduction of ACE inhibitors (ACEI) for the treatment of hypertension and the encouraging results of studies in diabetic rats with nephropathy have led to research in diabetic patients with microalbuminuria or normalbuminuria with or without hypertension which showed beneficial effects on renal function (Pedersen *et al*, 1988; Marre *et al*, 1988; Marre *et al*, 1991; Lewis *et al*, 1993; Viberti *et al*, 1994; the EUCLID Study Group, 1997). ACEI are now routinely used in the treatment of diabetic patients with proteinuria and those with microalbuminuria and hypertension. A meta-analysis of 100 studies of anti-hypertensive therapy has confirmed

the beneficial effects of ACEI on improving GFR and decreasing albumin excretion rates in nephropathy patients (Kasiske et al, 1993).

1.3.2.3. Prognosis in Diabetic Nephropathy

Once overt proteinuria is established most patients will reach ESRF within 10 years (Viberti, 1988; Ritz and Stephanski, 1996). Between 1975-1984, only 762 diabetic patients received RRT in the U.K.; less than 6% of new patients who received dialysis (Cameron and Challah, 1986). These findings prompted a study to assess whether the numbers being offered RRT had increased since 1984. The results indicated that one third of diabetic patients with ESRF died; the majority from untreated renal failure (Joint Working Party of the Renal Association *et al*, 1989). The recommendations from this study were that diabetic patients in ESRF should be offered dialysis and kidney transplantation.

The number of diabetic patients receiving RRT throughout the world varies considerably; in France in 1989, 6.9% of dialysis patients had diabetes in comparison to Japan with 40% in 1990 (Zmirou *et al*, 1992; Watanabe *et al*, 1993). However, the incidence of diabetic end-stage renal disease varies in different countries and in different ethnic groups within the same country (Ritz and Stephanski, 1996).

1.4 Other Microvascular Complications

1.4.1 Diabetic Retinopathy

Retinopathy is the microvascular disease which affects the small blood vessels of the retina and is due to damage to vascular endothelial cells. The disorder causes a variety of events which can be detected on the retina: new blood vessels form; "cotton wool spots" appear (these are the distended stumps of the ganglion-cell axons in the nerve cells supplying the retina) and exudates form which are composed of extracellular fluid, extracellular lipids and macrophages (Kohner *et al*, 1982). Retinopathy is graded according to the severity of the disease; background retinopathy includes a small number of exudates, "cotton wool spots", microaneurysms or small intra-retinal haemorrhages; maculopathy includes the above abnormalities spread through the macula and finally proliferative retinopathy includes the formation of new blood vessels outside the retina (Liang and Goldberg, 1980).

Retinopathy is recognised as one of the major causes of blindness in the western world. Visual loss from retinopathy is dependent on the duration of and age of onset of diabetes and the severity of the complication. After 10-15 years of Type 1 diabetes, 50% of patients will develop some degree of retinopathy. Patients with proliferative retinopathy have an increased risk of blindness due to macular oedema and vitreous haemorrhage. Macular oedema causes loss of visual acuity in greater numbers of diabetic patients than does vitreous haemorrhage; vitreous haemorrhage occurs when the

new blood vessels proliferate outside the retina into the vitreous cavity and haemorrhage occurs (Liang and Goldberg, 1980). However, when vitreous haemorrhage does occur, there is greater severity of visual loss.

In the EURODIAB IDDM Complications study, 46% of the 3,250 patients had retinopathy, 26% with mild background, 10% with moderate to severe background and 10% with proliferative (Sjolie *et al*, 1997). In Type 1 patients, nephropathy is usually accompanied by the other microvascular complications, retinopathy, which is often proliferative, and neuropathy. The EURODIAB study has also shown that the prevalence of retinopathy in patients with raised AER increased significantly with diastolic blood pressure above 85 mm Hg in comparison to people with normal albumin excretion rate (Stephenson *et al*, 1995). Another study in Sweden has shown that patients with retinopathy were older, the duration of diabetes was longer, the glycaemic control was worse, systolic blood pressure was higher than those people without retinopathy, they had higher albumin excretion levels and serum creatinine was elevated in patients with severe retinopathy (Agardh *et al*,1989). Both the Diabetes Control and Complications Trial (1993) and the United Kingdom Prospective Diabetes Study (1994) have demonstrated that intensive treatment to reduce blood glucose levels has a beneficial effect on reducing and preventing the development of retinopathy (American Diabetes Association, 1999).

1.4.2 Neuropathy

Long-term hyperglycaemia can affect the small blood vessels which supply the nervous system resulting in peripheral and / or autonomic neuropathy. There is a high morbidity from foot problems secondary to peripheral neuropathy and sudden death in diabetic patients may be associated with QT interval prolongation in people with autonomic neuropathy (Ward, 1999; Veglio *et al*, 1999). The mechanisms which cause neuropathy are not fully understood and as yet there is no treatment for neuropathy (Ward, 1999). The results of the EURODIAB Study have suggested a link between neuropathy, diastolic blood pressure and microalbuminuria (Tesfaye *et al*, 1996).

1.5 Cardiovascular Disease

Cardiovascular disease is a major cause of mortality in diabetic people (Kannel and McGee, 1979; Moss *et al*, 1991). Several factors contribute to the risk of cardiovascular disease developing in diabetic patients including hyperglycaemia, insulin resistance, presence of hypertension and abnormalities in coagulation, fibrinolysis and lipid profiles (Deckert *et al*, 1991; King and Wakasaki, 1999; Sowers and Lester, 1999). The presence of microalbuminuria in diabetic patients is predictive of early death from cardiovascular disease (Deckert *et al*, 1992). Diabetic women have an increased risk of developing and dying from cardiovascular disease, especially coronary heart disease (Manson *et al*, 1991; Moss et al, 1991). It has been postulated that an interaction between hyperglycaemia and oestradiol reduces the production of nitric oxide from endothelial cells (normally mediated by oestradiol), as nitric oxide may be associated with the protective effects of antioxidants, a reduction in the concentration of nitric oxide could lead to the development of atherosclerosis (Bruckdorfer, 1993; Sowers and Lester, 1999). Cardiovascular disease is associated with nephropathy and it has been suggested that the mechanisms underlying both conditions may be similar (Deckert *et al*, 1991).

Hypertension contributes to the morbidity and mortality from cardiovascular disease and nephropathy in patients with diabetes. Between 30-75% of diabetic complications are related to hypertension (Consensus Statement, 1993). Both Type 1 and Type 2 patients are at risk of developing

hypertension and as previously described much research has been performed to find the most effective anti-hypertensive treatments in nephropathy (Maki *et al*, 1995).

1.6 The Epidemiology of Diabetic Nephropathy

In a diabetic clinic population based study of 982 patients in Denmark, the prevalence of microalbuminuria was 22% and of macroalbuminuria was 19%. The patients with nephropathy were predominantly men and the prevalence was higher in people in whom diabetes had been diagnosed before 20 years of age (Parving *et al*, 1988). In the Wisconsin study, a large population based study, 18% of people on insulin treatment and 12% of patients not being treated with insulin had proteinuria, and the prevalence was found to increase with advancing age (Klein *et al*, 1988). The same study identified that proteinuria was associated with hypertension, systolic blood pressure, increasing severity of retinopathy and, as in the Danish study, was found predominantly in men (Klein *et al*, 1988).

The Rochester study demonstrated an incidence of nephropathy in non-insulin dependent diabetic people of 133 per 100,000 person-years and in insulin dependent patients: 170 per 100,000 person-years and showed the cumulative risk of Type 2 patients developing chronic renal failure within 10 years after diagnosis of proteinuria to be 11% (Humphrey *et al*, 1989).

The Steno study in Type 1 patients showed the cumulative incidence of nephropathy to be 45% after 40 years duration of diabetes and that there were two distinct incidence peaks for the onset of proteinuria at different durations of diabetes: the first occurred after 16 years and the second after 32 years (Andersen *et al*, 1983). The same study demonstrated a

prevalence of nephropathy of 21% after 20-25 years duration of diabetes which then declined to 10% after 40 years of diabetes (Andersen *et al*, 1983). In the EURODIAB study (Type 1 diabetic patients, n=3,250) the prevalence of macroalbuminuria ranged from 18-25% at five year intervals of diabetes duration and 19% of patients with a short duration of diabetes (between 1-5 years) had microalbuminuria (Stephenson *et al*, 1994).

The prevalence of macroalbuminuria has been shown to be higher in the USA (27%) than in Europe (12%) although prevalence of microalbuminuria was similar between the two continents (USA: 22 % and Europe: 25%) (Lloyd *et al*, 1996). There are differences in the incidence of end-stage renal failure (ESRF) in people of different ethnic groups within the same country: Pima Indians and Black people have a higher incidence of ESRF than White people in the USA, as do Aborigines in Australia and Indo-Asian people in the UK (Burden *et al*, 1992; Ritz and Stefanski, 1996). Perneger and co-workers found a 7.4 increased odds ratio for ESRF associated with Black people in the USA and Cowie and colleagues found a 2.6 greater incidence of ESRF in Black patients than in White people (Cowie *et al*, 1989; Perneger *et al*, 1994).

Although data from other centres are of a value, a key purpose of the research herein was to identify factors which could inform and improve clinical practice in a multi-ethnic community in the West Midlands region of the United Kingdom.

Chapter 2

Methodology and Statistical Analysis Used in This Research

2.1 Methods

2.1.1 Methods Used in The Mortality Study

This was a retrospective analysis of underlying causes of death in a sample of diabetic patients in comparison to non-diabetic people in the general population of Wolverhampton over a ten year period (1981-1990). Data on the diabetic sample and the non-diabetic general population (source: Office of Population Censuses and Surveys; SD 25, 1981-1990) were obtained from the Department of Public Health, Wolverhampton Health Authority; the data on deaths in diabetic people originated from death certificates (including all causes recorded in Parts I and II of the certificate) and were provided on a regular basis as typed lists of deaths. One hundred and ten deaths from diabetes in the OPCS data were not accounted for in the data on deaths in people with diabetes indicating that the data in diabetic patients did not include all of the diabetic population who died during the time period studied. For the analysis, information on all deaths with diabetes as the underlying cause was removed from the general population data to acquire a population free from diabetes, as far as could be ascertained from the death certificate.

Underlying causes of death were grouped and classified in accord with the recommendations of the World Health Organisation in the Ninth Revision of International Classification of Disease (World Health Organisation, 1977). These were then referred to as disease groups: cardiovascular (CVD) (ICD-9 390-459); this included all cardiac, vascular and cerebro-vascular disease;

respiratory (ICD-9 460-519), malignant neoplasms (ICD-9 140-208), liver (ICD-9 571), renal (ICD-9 580-589) and gastro-intestinal (G.I.) (ICD-9 520-579). All other causes of death were grouped together as "others". The disease group diabetes (ICD-9 250) (see Appendix 4) included diabetes, diabetic ketoacidosis (DKA), hyperosmolar coma (HOC), hypoglycaemia (HG), diabetic nephropathy and gangrene. Disease groups were subdivided into specific causes for deaths from diabetes, coronary heart disease ([CHD] and cerebrovascular accidents [CVA]).

Data obtained on diabetic patients from 1983 onwards were corrected according to the change in death certification known as "Rule 3", whereby any death certificate with pneumonia as the sole cause of death in Part I but with diabetes in Part II, had the Part II entry transferred to Part I i.e.: diabetes was recorded as the underlying cause of death (Williams, 1993). The general population data would have been subjected to "Rule 3" before publication.

Information on age, gender and underlying cause of death was collected for each year for both the diabetic sample and the general population. Initially, comparison was made of underlying causes of death in both groups over the ten year period. Then subjects were stratified according to age bands (15-34, 35-44, 45-54, 55-64, 65-74, 75-84 and > 85 years) and gender to permit comparisons based on age and to identify differences between men and women.

Figure 2.1: Flow diagram of mortality study

To fully assess the extent of coronary heart disease all cases with underlying causes of death recorded as ischaemic heart disease or myocardial infarction were added together in both groups of data. Deaths from the acute metabolic complications of diabetes, DKA, HOC, HG, were compared over the ten years as were those from diabetic nephropathy.

2.1.2 Methods Used to Study The Prevalence of and Factors Associated with Diabetic Nephropathy

This was a cross-sectional study using data collected retrospectively to determine the prevalence of diabetic nephropathy and accompanying diabetic complications in a cohort of diabetic patients with persistent proteinuria on albustix testing (*BM-Test-5L, Boehringer-Mannheim*; proteinuria = 5g l^{-1}) or raised serum creatinine concentrations (>120 μmol l^{-1}) referred from the diabetic clinic for nephrological assessment between 1987-1995. This study was a pragmatic piece of research based on routine clinical practice and therefore assessments which may otherwise have been performed as part of a structured research protocol were not performed unless they were part of routine medical care.

Patients were identified by consultant nephrologists and copies of letters generated after the assessment examination were kept in a separate file by medical secretaries to ensure that patients were identified for this study and none omitted from the analysis. Diabetic nephropathy was diagnosed on clinical grounds i.e.: presence of persistent proteinuria plus the presence of diabetic retinopathy or diagnosis was confirmed by renal biopsy where there was any doubt using the clinical definition. Data were collected from patients' hospital records on age, gender, diabetes history including complications and treatment, renal history, blood pressure measurements and anti-hypertensive therapy, smoking, family history of diabetes and physical parameters e.g.: body mass index, glycaemic control as measured by HbA1 and total blood cholesterol levels. Details of regular defaulting from routine diabetic clinic visits (i.e.: missing more than two consecutive clinic visits in more than one year since diagnosis of diabetes) were also collected. Patients were divided into Type 1 and Type 2 diabetes groups and a comparison was made between the two. Type 1 was defined as diagnosis before the age of 35 and requiring insulin treatment; Type 2 was defined as diagnosis after 35 years of age and treated with either diet, oral therapy or insulin treatment. For patients with proteinuria at diagnosis of diabetes the duration of diabetes was recorded as zero, only to give a numerical value, as it was accepted that Type 2 patients presenting with complications will have had diabetes for some time before diagnosis.

Renal function was assessed using serum creatinine levels and urinary protein excretion (as measured by 24 hour urine saves). Measurement of

creatinine clearance (the excretion of creatinine through the kidneys as measured using a 24 hour urine sample and a single blood sample taken during the time of the urine collection) would have given a more accurate assessment of glomerular filtration rate (Forrester *et al,* 1985). However, creatinine clearance was not performed in all nephropathy patients whereas serum creatinine concentrations were.

Retinopathy was defined as background (cotton wool spots and dot or blot haemorrhages) and severe retinopathy was defined as proliferative changes (e.g.: presence of new blood vessels) haemorrhage or loss of visual acuity not due to other conditions. Ischaemic heart disease was defined as documented evidence of angina symptoms or electrocardiographic evidence. Hypertension was defined as systolic pressure $\geq$ 140 mm Hg and / or diastolic pressure $\geq$ 90 mm Hg or receiving anti-hypertensive therapy as defined by the Working Group on Hypertension in Diabetes (1987). Severe peripheral vascular disease was defined as the presence of intermittent claudication or amputation.

Blood pressure was measured using a single sitting reading with a standard mercury Hawksley sphigmomanometer. Routine measurement of height and weight were made in the diabetic clinic; height if measured in inches was converted to centimetres, weight was measured in kilograms to permit calculation of body mass index.

All laboratory measurements were performed by the Clinical Chemistry Department, New Cross Hospital, Wolverhampton according to departmental standard operating procedures (Appendix 5). HbA1 was measured using the automated high performance liquid chromatography method by *Biomen (*in the same manner as HbA1c is now measured). Serum creatinine levels were measured using the Jaffe reaction (alkaline picrate) on *Bayer Diagnostics Chem 1* and latterly on *DAX*. Twenty four hour urinary protein excretion was measured using the pyrogallol red method on *Bayer Diagnostics RA1000* and total cholesterol was measured using the cholesterol oxidase method on *Bayer Diagnostics Chem 1* and latterly on *Dax* (Appendix 5).

Initially an analysis of all patients referred with renal disease was made to determine the point prevalence (prevalence at one point in time i.e.: nephrological assessment) of diabetic nephropathy and non-diabetic renal disease and the prevalence of diabetic complications in Type 1 and Type 2 patients. Comparisons were made between the groups to assess if there were differences in other parameters e.g: glycaemic control and blood pressure. A similar analysis was performed in Type 1 and 2 patients with nephropathy and in Type 2 nephropathy patients compared to Type 2 patients with non-diabetic renal disease.

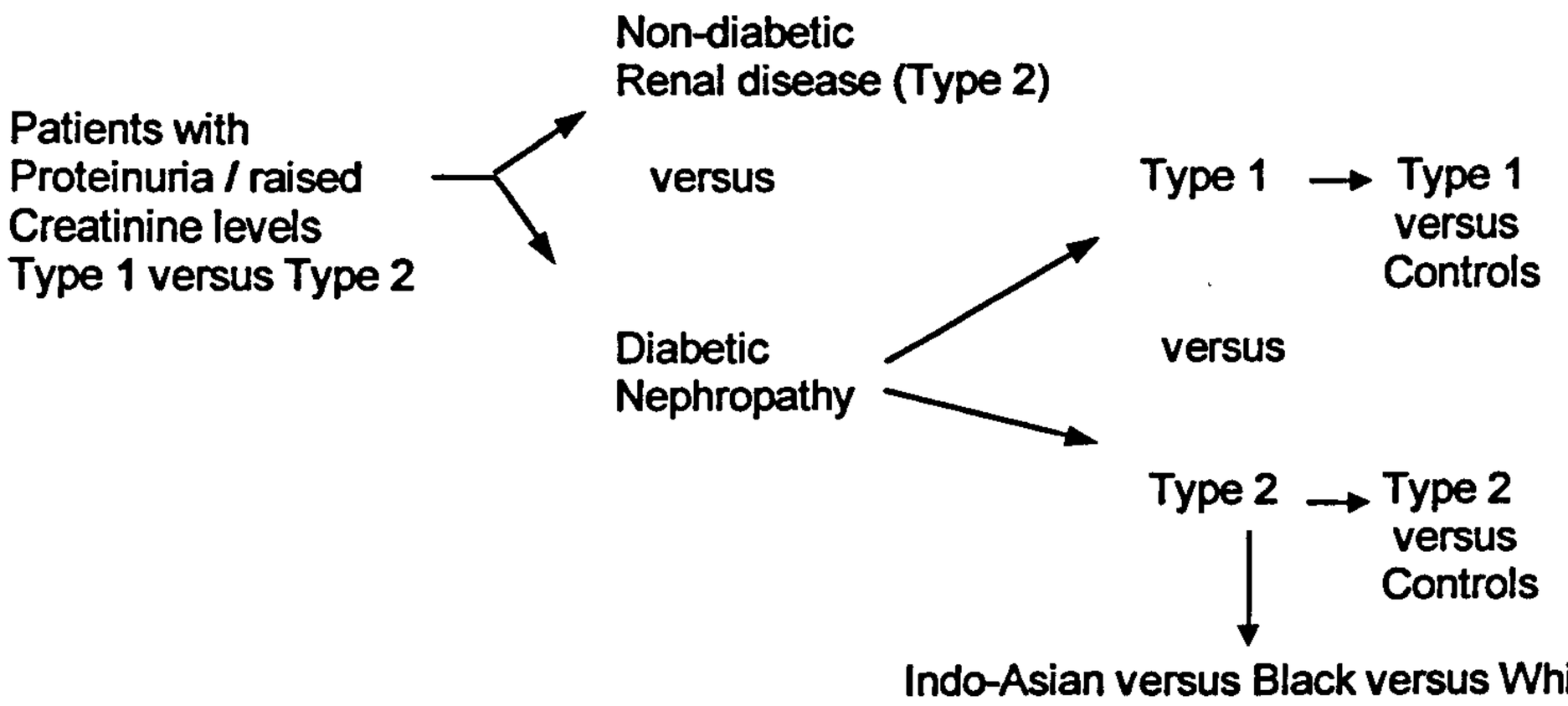


Figure 2.2: Flow diagram of diabetic nephropathy studies

Duration of diabetes to the onset of proteinuria, retinopathy and hypertension was also determined and compared in a subgroup of Type 1 and Type 2 patients with nephropathy who had all three complications, those without either retinopathy or hypertension were omitted. Only patients with a diagnosis of diabetes greater than three years were included, in order to exclude patients with established retinopathy and proteinuria before diagnosis of diabetes and to reduce the "unknown" duration of diabetes before diagnosis in Type 2 patients. A similar subgroup analysis was performed in the comparison of Type 2 patients with nephropathy and those with non-diabetic renal disease.

2.1.3 Methods Used in The Case-Controlled Comparisons of Nephropathy Patients with Type 1 and Type 2 Diabetes

Two case-controlled comparisons was made (1) between White Type 1 nephropathy patients and White Type 1 control subjects closely matched for age, gender, duration of diabetes and diabetic treatment (matching for age

and duration of diabetes was achieved by using bands of 5 years) and (2) between Type 2 patients with matched Type 2 control subjects, in this study matching also included ethnic groups. The large number of variables used to match the control subjects with nephropathy patients was to ensure that the groups were as similar as possible but also to remove these variables from the comparison (i.e.: to remove potentially confounding variables).

Control subjects were selected from 909 patients who attended the diabetic clinic for annual review examination between 1992-1995; the patients were identified by nursing staff and a medical secretary working in the diabetic clinic. The same type of data were collected from patients' hospital records in control subjects as previously described for patients with renal disease. Control subjects had no overt evidence of renal disease i.e.: they did not have proteinuria on "albustix" testing or raised serum creatinine levels. As routine testing for microalbuminuria was not performed in the diabetic clinic, it was not known whether control subjects had microalbuminuria. Only patients who had had serum creatinine concentrations measured were included in the control group and none had had creatinine clearance measured. The process of matching control subjects to those with nephropathy was performed after all data had been recorded on Smart databases, one for renal patients and the other for annual review patients. All computer data was checked for accuracy by comparison with the original collected written data.

In the study of Type 1 nephropathy patients versus controls, all nephropathy patients with serum creatinine concentrations > 200 μmol l^{-1} or who had reached ESRF were excluded. In the study of Type 2 nephropathy patients versus controls, all nephropathy patients with serum creatinine concentrations > 300 μmol l^{-1} or who had reached ESRF were excluded. Type 2 nephropathy patients were only included if they had a duration of diabetes greater than two years, had no retinopathy at presentation of diabetes and no proteinuria within the first three years after diagnosis of diabetes. These inclusion criteria were selected in order to identify a discrete group with nephropathy and decrease the likelihood that patients had undiagnosed diabetes for many years before diagnosis (although most Type 2 patients have a silent pre-diagnosis phase of diabetes). Control subjects in both of these studies had serum creatinine concentrations within the normal limits (60-120μmol l^{-1}). Type 1 controls were selected from 266 Type 1 patients and Type 2 controls from 643 Type 2 patients.

In both case-controlled studies comparisons were made between the groups which included diabetic complications, smoking history, defaulting from routine clinic attendance, blood pressures, anti-hypertensive therapy and HbA1 results for a four year period before nephrological referral. Subgroup comparisons were made between patients who were hypertensive and were or were not receiving anti-hypertensive therapy within and between the nephropathy and control groups.

2.1.4 Methods Used to Compare Type 2 Nephropathy Patients From Different Ethnic Groups

Type 2 patients with nephropathy were divided into three groups dependent on ethnic origin; White (defined as people with genetic origins in Europe, irrespective of place of birth), Indo-Asian (defined as people with genetic origins from the Indian subcontinent, irrespective of place of birth) and Black (defined as people with genetic origins from Africa irrespective of place of birth) and matched according to age, gender, duration of diabetes and diabetes treatment. For the initial analysis, patients on renal replacement therapy or with serum creatinine levels greater than 300 μmol l^{-1} were omitted from the analysis to remove bias due to advanced disease. A further analysis was performed to validate the results of Chi^2 tests by using larger numbers of patients; this included patients with serum creatinine concentrations above 300 μmol l^{-1} and those on RRT.

A point prevalence i.e.: prevalence at a single examination (nephrological assessment) was determined for diabetes complications and other factors that may be associated with nephropathy in the different ethnic groups.

Duration of diabetes to the onset of proteinuria, retinopathy and hypertension were also determined and compared between the groups to assess whether there were any differences in development of these complications.

A comparison was made between patients with treated and untreated hypertension to determine whether there were factors which contributed towards lack of anti-hypertensive therapy which were specific to any of the ethnic groups.

2.1.5 Methods Used to Assess the Effectiveness of Routine Clinical Care on Survival (Preventative Study)

Patients with nephropathy referred for nephrological assessment were followed up over a maximum period of twelve years (1987-1998) to assess the effect of normal clinical practice on survival. Normal clinical practice was defined as treatment determined by consultant nephrologists for individual patients and individual medical problems on an "intention to treat" basis; there was no written pre-determined protocol for treatment which could be followed by junior medical staff, although individual cases were discussed with the consultant. Follow up was defined as regular attendance at the out-patient clinic, though the number of visits per year varied according to the clinical condition of the patient. Patients were excluded from the study if they were receiving renal replacement therapy at the study onset or if they had only one functioning kidney, as this may have affected the rate of decline of renal function. As patients were referred at different times over the twelve year period, length of follow up time varied from patient to patient.

Data were collected from patients' hospital records on blood pressure measurements, serum creatinine concentrations, urinary protein levels, and

glycaemic control as measured by HbA1 (both pre- and post-referral), over the time period since referral, as well as demographic data. Information on end points was also collected. The end points were: survival without reaching ESRF, need for renal replacement therapy (RRT), survival on RRT, death, death following RRT and lost to follow up. Cause of death was also recorded. In some cases, the causes of death could not be determined due to inability to locate patients' hospital records after death. Data collected at referral were the baseline data used to compare data collected annually in subsequent years up to year 7, from this time point numbers of patients were too low to reliably perform statistical analysis.

A number of different parameters were examined to find the potential effect of these on survival. Hypertension was defined as a systolic pressure > 140 mm Hg or diastolic pressure > 90 mm Hg and this decided the "need for treatment". Age at renal referral, duration of diabetes, time from onset of overt proteinuria to renal referral, duration of diabetes until onset of proteinuria, systolic and diastolic blood pressures, serum creatinine, 24 hour urinary protein excretion, anti-hypertensive treatment (as measured by point scale, see Appendix 1) and presence or absence of this at baseline. All of the above were compared in all of the Type 1 and Type 2 groups and, in addition, two other analyses were performed: 1) in three ethnic groups: Indo-Asian (people with ethnic origins in the Indian subcontinent whether born there or elsewhere): Black (people with ethnic origins in either Africa or the Caribbean, irrespective of place of birth): and White patients and 2) a subgroup analysis of Type 1 and Type 2 patients with serum creatinine

levels below 120 μmol l^{-1} (upper limit of normal) to remove bias due to advanced renal disease.

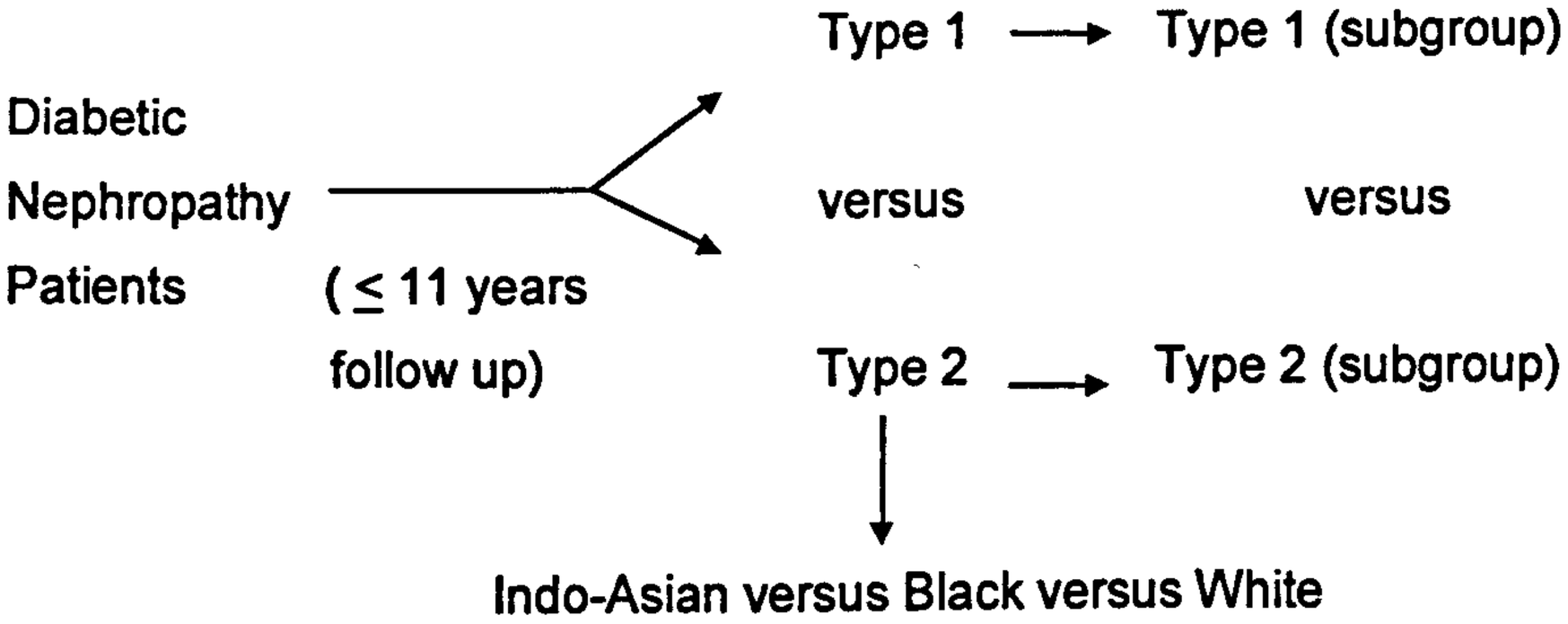


Figure 2.3: Flow diagram of study assessing the effectiveness of routine clinical care and survival analysis in nephropathy patients.

2.2 Statistical Analysis

2.2.1 Analysis of Mortality Study

Analysis was restricted to a direct comparison using Chi^2 tests of proportion of deaths in diabetic compared to non-diabetic people and then according to age stratification and gender. The odds ratio (OR) was calculated as an estimate of the relative risk of one group dying from a specific cause in comparison to the other group; the actual relative risk could not be performed as the data did not include the full population of deaths in people with diabetes (Silman, 1995; Gordis, 1996).

The calculation used for the odds ratio was: $OR = \frac{axd}{bxc}$

where a= number of diabetic people dying from a specific underlying cause

b= number of non-diabetic people dying from the same specific cause

c= number of diabetic people dying from other underlying causes

d= number of non-diabetic people dying from other underlying causes

Confidence intervals were calculated for the odds ratios using Woolf's Method (Silman, 1995). For 95% confidence intervals (C.I.) the following calculation was used:

$$95\%\ C.\ I. = \exp\left(\log_e OR \pm 1.96 \sqrt{1/a+1/b+1/c+1/d}\right)$$

where a, b, c, d were defined as above.

e.g: for deaths with cardiovascular disease as the underlying cause.

Lower 95% C.I.:

$= \exp (\log_e OR - 1.96\sqrt{1/a+1/b+1/c+1/d})$

$= \exp (\log 1.27 - 1.96 X 0.0545)$

$= \exp (0.238-0.107)$

$= \exp (0.131)$

$= \underline{1.14}$

Upper 95% C.I. :

$= \exp (\log_e OR + 1.96\sqrt{1/a+1/b+1/c+1/d})$

$= \exp (\log 1.27 + 1.96 X 0.0545)$

$= \exp (0.238+0.107)$

$= \exp (0.345)$

$= \underline{1.41}$ Therefore, 95% Confidence Interval = (1.14, 1.41)

The odds ratio has logarithmic properties, therefore, it does not lie mathematically in the centre of the lower and upper confidence intervals (Silman, 1995).

To achieve 80% power for this study with a significance level of $P \leq 0.05$, the sample size was calculated using the following formula for two proportions: $N=8[p_1 (1- p_1) + p_2 (1- p_2)]/(p_1-p_2)^2$ per group (Lowe, 1993). The proportion of deaths from CVD in diabetic and non-diabetic people in the Aberdeen study were used to give an indication of likely proportions of deaths from the same cause in this study: deaths from CVD in diabetic people (56%) and non-diabetic people (41%) (Wong *et al*, 1991):

$N= 8[(0.56)(0.44)+(0.41)(0.59)]/(0.56-0.41)^2$ per group

N=174 per group. This result was confirmed using a computerised sample size calculator (Brant, 1999).

Once data was collected the power of the study was determined using the proportion of deaths from CVD in each group at a statistical power $P \leq 0.05$ for a two sided test. However, as the numbers in each group were not equal, the harmonic mean was calculated using the formula:

$$N_h = \frac{2(N_1 N_2)}{N_1 + N_2}$$

and substituted for the sample size using a computerised power calculator, (Brant, 1999).

2.2.2 Analysis of Diabetic Nephropathy Studies

Data analysis was performed using SPSS and Excel statistics packages. Descriptive statistics [mean, (±standard deviation), median, minimum, maximum and inter-quartile ranges] were obtained and presented in tables or as boxplots (box-and whisker-plots). The chosen level of statistical significance was $P \leq 0.05$ in one and two-tailed tests. The statistical power of the studies was determined once the number of patients in each of the groups were known, at the start of the research the final numbers could be not be anticipated as a pilot study was not performed.

Quantitative data were analysed using Student t test for parametric (e.g.: age or duration of diabetes) or Mann-Whitney U test for non-parametric (e.g.: serum creatinine concentrations) where two sets of data were compared. One-way analysis of variance was used if there were more than two sets of data (e.g.: HbA1 over four years) to determine how reliable the mean differences were (Tabachnick and Fidell, 1996). Scatterplots were produced to assess whether the relationship between two variables was linear (e.g.: time to development of retinopathy and proteinuria). The strength of the linear relationship between two variables was then compared, after any outlying points (maximum n=3) were removed, using the Pearson correlation coefficient (Kinnear and Gray, 1994; Tabachnick and Fidell, 1996; SPSS inc.,1999). Linear regression was used to determine whether one variable could predict another and the multiple correlation coefficient (R^2) was used to determine the variance of the dependent variable predicted by the independent variable (e.g.: age as a predictive variable for diastolic blood pressure) (Johnson and Tsui, 1998).

Multiple regression analysis was used to determine the predictive relationship between several independent variables and one dependent variable (e.g.: time to onset of hypertension and retinopathy, as a combination of independent variables, to determine whether together they increased the prediction of time to onset of proteinuria). Multiple regression analysis was dependent on the linear relationship (ascertained by scatterplots) between the dependent variable and the independent variables which were either continuous or dichotomous variables (Tabachnick and

Fidell, 1996). Sequential and stepwise multiple regression analyses were also performed (time to onset of hypertension and retinopathy as predictors of onset of proteinuria were also used in sequential and stepwise multiple regression analysis). The sequential multiple regression allowed the addition of different independent variables in a particular order (with the variables considered to have the greatest predictive value first) whereas in stepwise multiple regression the order in which variables were added into the regression equation was dependent on statistical considerations alone (determined by the statistical software and not necessarily in the order the researcher would have chosen). Variables were also removed when these were found to have no predictive effect during the stepwise multiple regression technique (Tabachnick and Fidell, 1996; SPSS inc.,1999). The removal of outlying results not only increased the accuracy of the Pearson correlation coefficient but also the various regression techniques used, as all forms of regression analysis are very sensitive to outlying results (Tabachnick and Fidell, 1996).

Logistic regression analysis was used to determine whether a set of variables could predict a single variable (e.g.: variables such as defaulting from clinic visits, anti-hypertensive therapy, smoking status, gender, systolic and diastolic blood pressures were assessed as predictors of serum creatinine). This technique was used in preference to other methods of multivariate analysis because it was more flexible i.e.: predictive variables do not have to have a linear relationship with the dependent variable or be of normal distribution and no assumption was made concerning equality of the

variance plus any type of variable (dichotomous, discrete or continuous) could be used (Tabachnick and Fidell, 1996). Both direct (all variables added simultaneously) and sequential (variables added one after the other) logistic analysis were performed.

The odds ratio was calculated as an estimate of the relative risk of the presence of different variables for developing nephropathy; the actual relative risk could not be performed as these were case controlled studies (Silman, 1995; Gordis, 1996).

The power of the various studies at $P \leq 0.05$ were determined after data collection using a computerised power calculator (Brant, 1999; UCLA, 1999). Where the standard deviations of two populations (σ) were needed to calculate the power, the harmonic mean of σ was calculated.

All qualitative data e.g.: presence of complications, gender or smoking status, were changed into dichotomous variables by assigning either yes = 1 or no = 0 in order to perform Chi^2 tests to assess differences between groups (Tabachnick and Fidell, 1996). Where the expected frequencies obtained during Chi^2 test were < 5, Fischer's exact test was performed in 2x2 contingency tables only; if the number of variables were such that the degrees of freedom were >1, Fischer's exact test was not performed (SPSS Inc, 1999).

2.2.3 Analysis of the Effectiveness of Routine Clinical Care and Survival Analysis (Preventative Strategies)

Descriptive statistics were determined at baseline (nephrological assessment examination), repeated measures analysis of variance was used to determine differences in mean values between the groups and within the groups, when parameters from different years were compared (e.g.: serum creatinine concentrations, systolic and diastolic blood pressures). Chi^2 test were used to compare categorical descriptive variables.

Cumulative survival was determined using Kaplin-Meier survival curves and the difference in the equality of the survival distribution was compared using the Logrank test. The mean and median values were presented along with standard error and 95% confidence intervals. End points were death, survival, lost to follow up (patient stopped attending nephrology and diabetic clinics) and renal replacement therapy (RRT). Data on patients who survived or were lost to follow up were censored as they had not reached the other two end points.

The Kaplin–Meier estimate of survival is the successive overall probability of survival over a period of time, usually expressed in graph form, where at the baseline time point all subjects are assumed to be still living i.e.: at time=0, there is 100% survival (Parmar and Machin, 1995). The Logrank test was used to compare two survival curves to determine whether the survival distributions of the curves were equal i.e.: was there a statistical difference in

survival between the curves? Prior to calculation of this, all data were ranked with censored data having a higher rank than deaths. The Logrank test involved calculating observed and expected deaths, a formula similar to the Chi^2 tests: -

$$\chi^2_{Logrank} = \frac{(O_A-E_A)^2}{E_A} + \frac{(O_B-E_B)^2}{E_B}$$ where A =data set 1 and B = data set 2.

Degrees of freedom =1 (Parmar and Machin, 1995).

The Cox proportional hazard model was used to determine the effect of different variables as a hazard for survival in the form of hazard ratios (prognostic significance). Hazards which are not constant i.e.: they can vary within a patient group, are proportional hazards to survival (whereas hazard rate is assumed to be constant). The Cox proportional hazard model is a model which allows non-constant variables to be assessed in terms of hazard to survival that does not make assumptions about the distribution of the hazard in the groups tested (Pamar and Machin, 1995). The only assumption is that the hazard is proportional in each group throughout the study. The hazard ratio for the Cox proportional model when comparing two groups of patients equals $T:h_x=\exp(\beta x)$, where T represents the model which is being examined and x represents the difference between the two groups, which is equivalent to HR= exp (β), where HR is the hazard ratio. The Cox model uses regression analysis as the formula used to determine HR = exp (β) is in the form of a straight–line regression model (Pamar and Machin, 1995; Johnson and Tsui, 1998). An HR<1 signifies a better risk (reduced hazard) and HR>1 signifies increased hazard. Data were adjusted for various characteristics (to remove any effect due to these variables) prior to

analysis. All hazard ratios greater than 1, were presented in table form, along with standard error, 95% confidence intervals and level of statistical significance.

For the survival analysis, the sample size for the whole study was calculated based on a number of assumptions:

- The anticipated difference between the groups: $\delta_0 = \pi_{Type\ 1} - \pi_{Type\ 2} = 0$ for H_o.
- A fixed survival time was used: 5 years (1826 days)
- The statistical significance = 0.05
- The power of the study = 80%

As the only available results of previous studies were in patients who were receiving RRT, it was decided that it would not be appropriate to use these to calculate the numbers required in a study of patients who were not on dialysis.

Therefore, based on the assumptions that

- both Type 1 and Type 2 patients were at risk of death from similar causes e.g.: cardiovascular disease
- the rate of deterioration of renal function is similar in both groups (Beisenbach *et al*, 1994)
- Type 2 patients were older
- Type 2 patients were less likely to receive RRT
- Cumulative survival in Type 2 patients would be lower than in Type 1 patients over 5 years

it was assumed that an anticipated difference in survival between the groups would be δ=0.20 with 50% of Type 1 patients and 30% of Type 2 patients surviving for 5 years, giving a required sample size of 91 in each group, with $P \leq 0.05$ and 80% power (Parmar and Machin, 1996).

Literature searches were performed using Medline, the Cochrane database, the Citation Index and the Index Medicus. Some references were obtained from the bibliographies of published articles.

A system for allocating points to anti-hypertensive medication and dosages is described fully in Appendix 1.

2.3 Ethical Considerations

This study was approved by the Wolverhampton District Research Ethics Committee in 1992. Patients' consent was not obtained as all information was extracted from hospital records and all interventive treatments were part of normal clinical care rather than as part of a research project. For the same reason General Practitioners were not informed that their patients were participating. Patient confidentiality was maintained throughout the study by the use of identification codes rather than patients' names during the data collection process and when data were recorded on computer databases. A separate written record of patients names and identification codes was maintained to allow follow up for the survival analysis.

2.4 The Aims of This Study

The overall aims of this research were:

1 To determine whether diabetic nephropathy is a significant cause of mortality in people with diabetes in Wolverhampton.

2 To identify factors which were associated diabetic nephropathy .

3 To identify factors which place diabetic patients at risk of developing nephropathy.

4 To assess how effective routine clinical care was on survival in patients with nephropathy, following nephrological referral and follow up.

More specific aims and the hypotheses to be tested are identified in subsequent chapters.

Chapter 3

Mortality in Diabetic and Non-Diabetic People in Wolverhampton, 1981-1990

3.1 Introduction

Mortality and morbidity from diabetes mellitus have long been recognised as a major health problem. The cardiovascular complications of diabetes are known to contribute to a significant proportion of deaths in diabetic patients (Kannel and McGee, 1979; Turnbridge *et al*, 1981; Moss *et al*, 1991). Research has shown that the majority of cardiovascular deaths are due to coronary heart disease (CHD) and that diabetic patients have an increased relative risk of developing CHD in comparison to non diabetics (Manson *et al*, 1991; Folsom *et al*, 1997). End stage renal failure (ESRF) secondary to diabetic nephropathy has been identified as a microvascular complication that does not necessarily result in death. In 1989, recommendations were made that diabetic patients in ESRF should be given renal replacement therapy at an earlier stage of renal failure than non-diabetics (Joint Working Party British Diabetic Association *et al*, 1989). Cardiovascular disease is still the major cause of death in diabetic patients and a world wide study has shown excess mortality in patients with both types of diabetes, proteinuria and hypertension (de Grauw *et al*, 1995; Wang *et al*, 1996).

Wolverhampton is a large industrial town in the West Midlands with a population of 242,190 recorded at the last census of mixed ethnic groups, primarily White (81.4%), Indian (11.4%) and Black-Caribbean (4.1%) (Office of Population Censuses and Surveys, 1992). The question as to whether the results of studies in diabetic patients in other towns, cities or countries apply

in Wolverhampton has not been answered. The aim of this study was to provide retrospectively an overview of mortality in the diabetic population of Wolverhampton in comparison to the general population by the use of information on deaths in diabetic patients between 1981 and 1990. This study will act as an introduction to further research investigating factors which are either predictive or put patients at risk of developing nephropathy in Wolverhampton.

3.2 Objectives

The objectives of this study were:

(a) To determine whether there was a difference in the underlying causes of death recorded in diabetic and non-diabetic people.

(b) To assess whether there were differences in the underlying causes of death between men and women with and without diabetes.

(c) To assess whether there were differences in the underlying causes of death between people with and without diabetes of different age groups.

(d) To determine the relative contribution of nephropathy to mortality in diabetic patients in Wolverhampton.

3.3 Hypotheses

1 H_0: There was no difference in underlying causes of death between people with diabetes and those without diabetes.

H_1: There was a difference in underlying causes of death between people with diabetes and those without diabetes.

2 H_0: There was no difference in underlying causes of death between men and women with diabetes.

H_1: There was a difference in underlying causes of death between men and women with diabetes.

3 H_0: There was no difference in underlying causes of death in people with diabetes of different ages.

H_1: There was a difference in underlying causes of death in people with diabetes of different ages.

4 H_0: Diabetic nephropathy was not a major underlying cause of death in people with diabetes.

H_1: Diabetic nephropathy was a major underlying cause of death in people with diabetes.

3.4 Methods

Details of deaths (as recorded in Parts I and II of the death certificate)(See Appendix 3) in diabetic patients and the general population in Wolverhampton over a ten-year period (1981-1990) were supplied by the Department of Public Health, Wolverhampton Health Authority; the data on diabetic people originated from death certificates (including all causes recorded in Parts I and II of the certificate) from the office of the Registrar of Births, Deaths and Marriages, Wolverhampton.

Underlying causes of death were coded and grouped in accord with that recommended by the World Health Organisation in the Ninth Revision of the International Classification of Disease (World Health Organisation, 1977). These were then referred to as disease groups: cardiovascular (CVD) (ICD-9 390-459); this included all cardiac, vascular and cerebro-vascular disease; respiratory (ICD-9 460-519), malignant neoplasms (ICD-9 140-208), liver (ICD-9 571), renal (ICD-9 580-589) and gastro-intestinal (G.I.) (ICD-9 520-579). All other causes of death were grouped together as "others". The disease group diabetes (ICD-9 250) (see Appendix 4) included diabetes, diabetic ketoacidosis (DKA), hyperosmolar coma (HOC), hypoglycaemia (HG), diabetic nephropathy and gangrene. Disease groups were subdivided into specific causes for diabetes and coronary heart disease ([CHD] and cerebrovascular accidents [CVA]).

The underlying causes of death in diabetic people were compared to underlying causes of death in the general population of Wolverhampton from data published by the Office of Population Censuses and Surveys over the same period (OPCS: SD 25 1981-1990). One hundred and ten deaths from diabetes in the OPCS data were not accounted for in the data on deaths in people with diabetes. Therefore, this data in diabetic patients did not include all of the diabetic population who died during the time period studied. For the analysis, information on all deaths with diabetes as the underlying causes was removed from the general population data to acquire a population free from diabetes, as far as could be ascertained from the underlying causes of death. Information on age, gender and underlying cause of death was collected for each year for both the diabetic sample and the non-diabetic general population.

Data obtained on diabetic patients from 1983 onwards were corrected according to the change in death certification known as "Rule 3", whereby any death certificate with pneumonia as the sole cause of death in Part I but with diabetes in Part II, had the Part II entry transferred to Part I i.e.: diabetes was recorded as the underlying cause of death (Williams, 1993). The general population data would have been subjected to "Rule 3" before publication.

Comparisons were made of all underlying causes of death in all subjects over the ten years followed by a comparison based on gender and age

stratification to identify significant differences between men and women and in different age bands. To fully assess the extent of coronary heart disease all cases with underlying causes of death recorded as ischaemic heart disease or myocardial infarction were added together in both groups of data. Deaths from the acute metabolic complications of diabetes, DKA, HOC, HG, were compared over the ten years as were those from diabetic nephropathy.

Chi ^{2} tests were used to compare differences between the groups. The estimated relative risk of one group dying from a specific cause in comparison to the other group was determined using the odds ratio (OR). The level of significance was $P \leq 0.05$ for one and two tailed tests.

3.5 Results

Data on 28,411 deaths in the Wolverhampton population between 1981-1990 were used in this study; 26,990 deaths were classified as having occurred in non-diabetic people and 1,421 in people with diabetes. The non-diabetic group was composed of 13,835 men (51%) and 13,155 women (49%) in comparison to 680 (48%) men and 741 (52%) women in the diabetic group. There were significantly more women in the diabetic group (P <0.05).

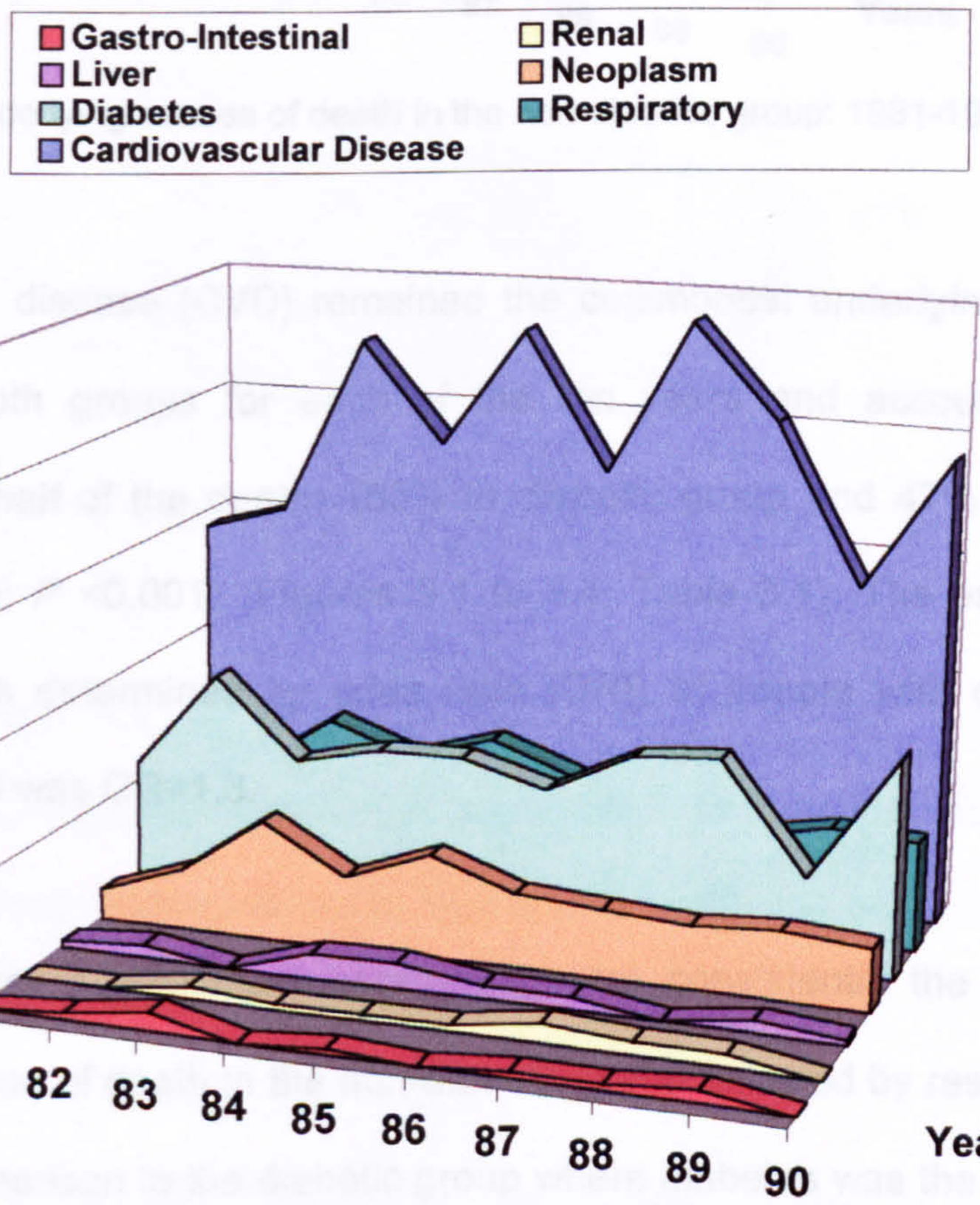


Figure 3.1: Underlying causes of death in the diabetic group:1981-1990

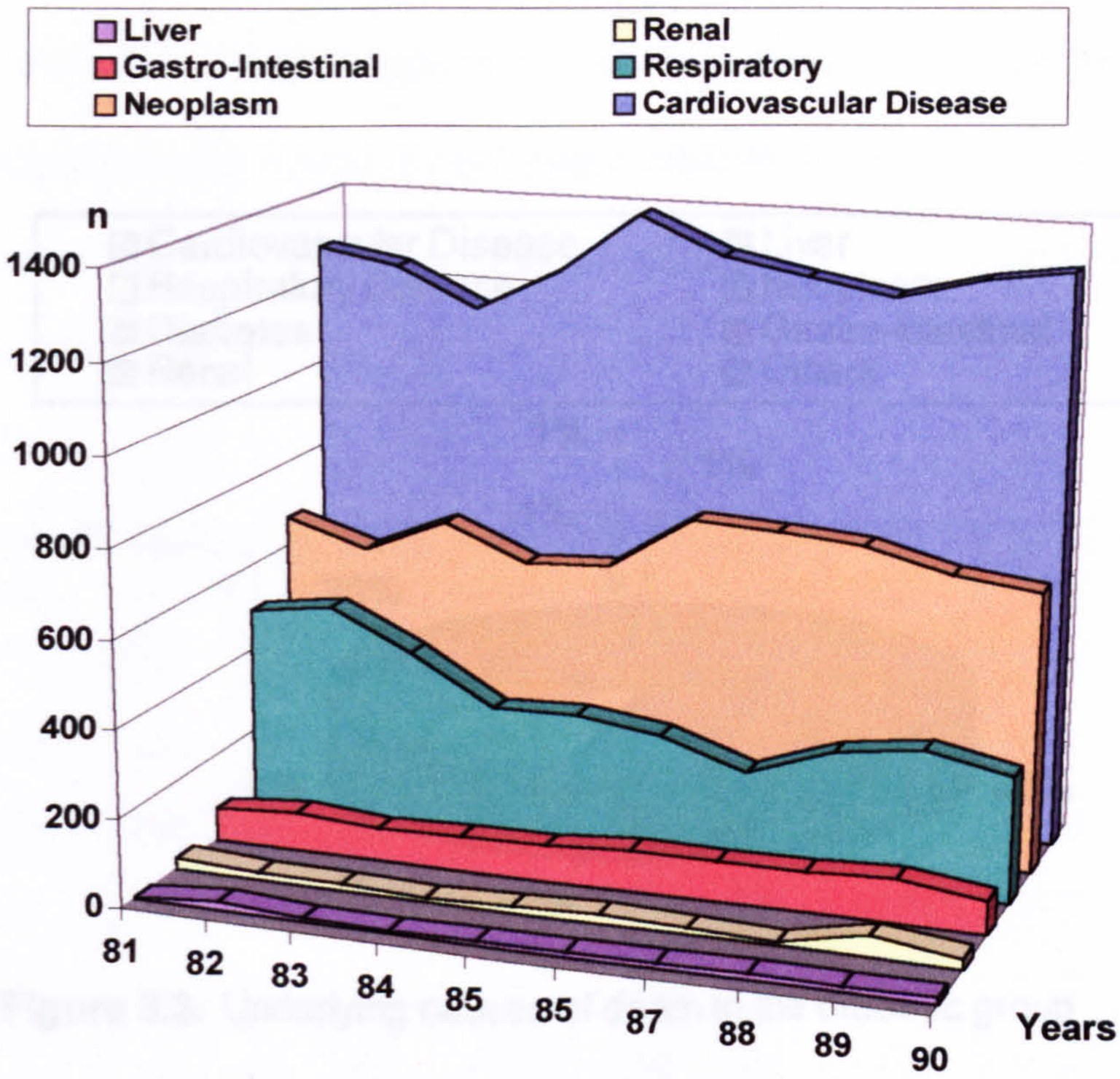


Figure 3.2: Underlying causes of death in the non-diabetic group: 1981-1990

Cardiovascular disease (CVD) remained the commonest underlying cause of death in both groups for each of the ten years and accounted for approximately half of the deaths (53% in diabetic group and 47% in non-diabetic people; P <0.001) (Figures 3.1 to 3.4, Table 3.1). The estimated relative risk (as determined by odds ratio [OR]) of people with diabetes dying from CVD was OR=1.3.

Over the ten years, malignant neoplasms were consistently the second commonest cause of death in the non-diabetic group followed by respiratory disease in comparison to the diabetic group where diabetes was the second commonest cause also followed by respiratory disease (Figures 3.1 and 3.2).

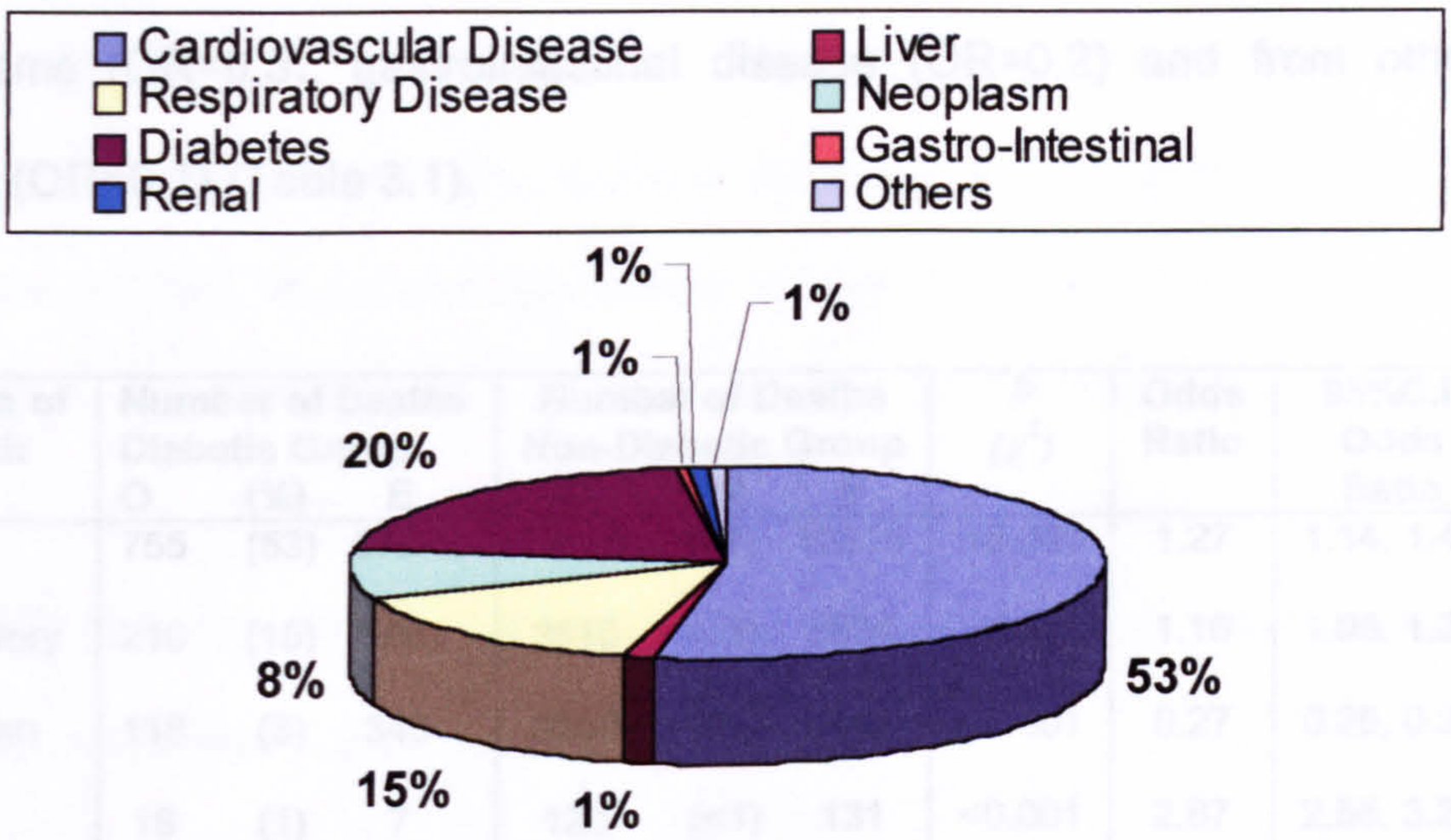


Figure 3.3: Underlying causes of death in the diabetic group

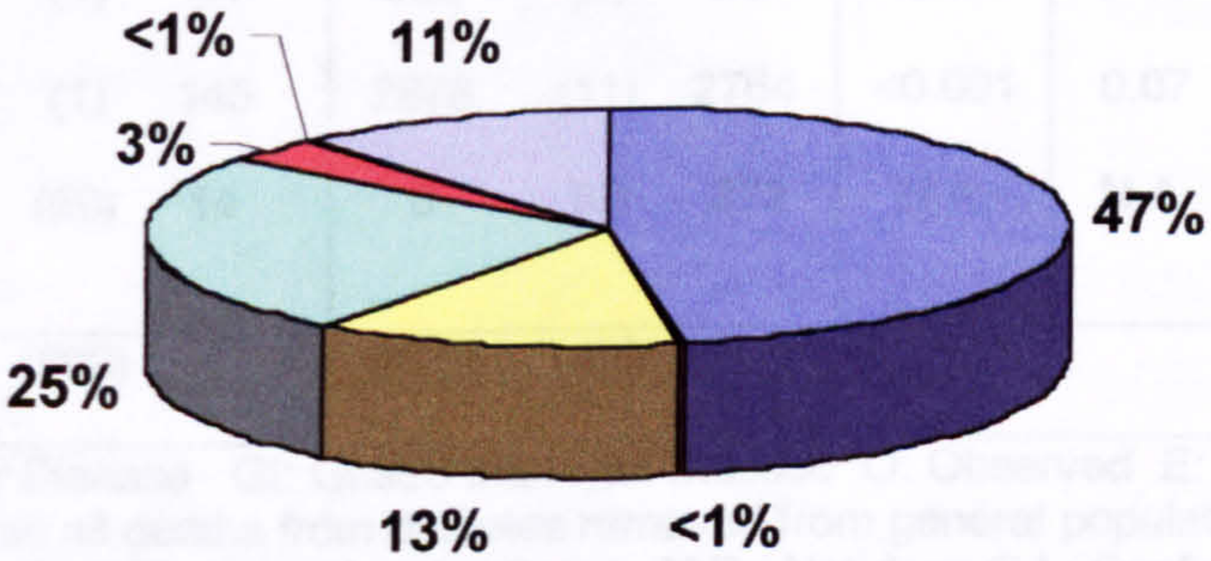


Figure 3.4: Underlying causes of death in the non-diabetic group

The highest proportion of deaths in diabetic patients were from CVD, diabetes and respiratory disease in comparison to non-diabetic people where the highest proportion were from CVD, malignant neoplasms and respiratory disease (Figures 3.3 and 3.4; Table 3.1).

There was no significant difference in deaths from respiratory and renal diseases between the groups (Table 3.1). The estimated relative risk of

death from liver disease was increased in people with diabetes (OR=2.9) whereas there was a reduced estimated relative risk of dying from malignant neoplasms (OR=0.3), gastrointestinal disease (OR=0.2) and from other causes (OR=0.1) (Table 3.1).

Causes of Death	Number of Deaths Diabetic Group O (%) E	Number of Deaths Non-Diabetic Group O (%) E	*P* (χ^2)	Odds Ratio	95%C.I. Odds Ratio
CVD	755 (53) 675	12735 (47) 12815	<0.001	1.27	1.14, 1.41
Respiratory	210 (15) 186	3510 (13) 3534	N.S.	1.16	1.03, 1.39
Neoplasm	118 (8) 340	6688 (25) 6466	<0.001	0.27	0.25, 0.36
Liver	18 (1) 7	120 (<1) 131	<0.001	2.87	2.55, 3.29
Renal	12 (1) 10	197 (<1) 199	N.S.	1.16	0.67, 2.15
G.I.	9 (1) 44	862 (3) 827	<0.001	0.19	0.10, 0.37
Others	12 (1) 145	2878 (11) 2764	<0.001	0.07	0.04, 0.12
Diabetes	287 (20) 14	0 (0) 272	N.A.	N.A.	N.A.
Totals	1421 (100)	26,990 (100)			

CVD: Cardiovascular Disease GI: Gastro-intestinal Disease O: Observed E: Expected
N.A.: Not applicable as all deaths from diabetes removed from general population data
Level of significance: $P \leq 0.05$ N.S.: Not significant N.D.: Not done C.I.: Confidence Interval

Table 3.1: Underlying causes of death in people with diabetes and those without diabetes.

Using the proportion of deaths from CVD in each group, the sample size was recalculated to confirm the power of the study, as the numbers in the groups were unequal, the harmonic mean of n was determined (2700) and used in the calculation. At a significance level of $P \leq 0.05$, the power of the study was calculated at 99% (Brant, 1999).

When total deaths from CVD were compared in women, there was no difference between the groups (diabetic: n=376 [51%] versus non-diabetic: n=6367 [48%]; *P*=N.S.). However, the age stratified comparison demonstrated that a higher proportion of deaths in female diabetic patients were due to CVD in certain age bands: 15-34 (however, actual numbers were very small; OR=5.3, 95% CI: 0.45, 62.80), 45-54 (OR=4.1) and 55-64 (OR=2.5) years (Table 3.2). A smaller proportion of diabetic women died from CVD between 35-44 years and from 75 years of age onwards than non-diabetic women (Table 3.2).

Age Group (years)	Number of Deaths from CVD Diabetic Women			Number of Deaths From CVD Non-Diabetic Women			*P* (χ^2)	Odds Ratio	95%C.I. Odds Ratio
	O	(%)	E	O	(%)	E			
>85	62	(45)	85	1849	(62)	1826	<0.001	0.50	0.35, 0.70
75-84	128	(47)	166	2591	(62)	2553	<0.001	0.55	0.43, 0.70
65-74	121	(56)	108	1409	(54)	1413	N.S.	1.08	0.81, 1.43
55-64	52	(60)	34	484	(38)	502	<0.001	2.47	1.59, 3.85
45-54	11	(58)	5	108	(25)	114	<0.001	4.09	1.60, 10.43
35-44	0	(0)	3	32	(84)	29	N.A.	N.A.	N.A.
15-34	2	(67)	1	15	(27)	16	N.A.	5.33	0.45, 62.80
Totals	376			6367					

O: Observed E: Expected %: Percentage of total deaths in each age band N.S.: Not significant
Level of significance: $P \leq 0.05$ N.A.: Not applicable (expected frequency < 5 for χ^2 test)
N.D.: Not done C.I.: Confidence Interval

Table 3.2: Deaths in women from cardiovascular disease

Three hundred and seventy-six (54%) diabetic men died from CVD in comparison to 6,312 (46%) non-diabetic men (*P* <0.001). A higher proportion of men with diabetes between the ages of 45-54 (OR=2.4) and

55-64 years (OR=1.8) died from CVD than non-diabetic men (Table 3.3). These results suggest that women with diabetes between the ages of 45-64 years have a greater estimated relative risk of dying from CVD than diabetic men of the same age.

Age Group (years)	Number of Deaths from CVD Diabetic Men O	(%)	E	Number of Deaths from CVD Non-Diabetic Men O	(%)	E	*P* (χ^2)	Odds Ratio	95%C.I. Odds Ratio
>85	16	(41)	20	574	(51)	570	N.S.	0.67	0.35, 1.28
75-84	103	(50)	107	2021	(53)	2017	N.S.	1.02	0.77, 1.35
65-74	145	(56)	132	2017	(50)	2086	N.S.	1.32	1.02, 1.69
55-64	80	(63)	63	1127	(49)	1144	<0.005	1.77	1.23, 2.57
45-54	24	(73)	18	411	(53)	476	<0.001	2.37	1.09, 5.17
35-44	8	(53)	8	116	(54)	116	N.S.	0.97	0.33, 2.81
15-34	0	(0)	0	46	(46)	N.A.	N.A.	N.A.	N.A.
Totals	376			6312					

O: Observed E: Expected %: Percentage of total deaths in each age band N.S.: Not significant
Level of significance: $P \leq 0.05$ N.A.: Not applicable N.D.: Not done CI: Confidence Interval

Table 3.3: Deaths in men from cardiovascular disease

When CVD was divided into deaths from CVA and CHD, there was no difference between the groups with CVA as the underlying cause of death: diabetic: (n=149, 11%) and non-diabetic (n = 2,998, 11%) (*P*=N.S.). Diabetic men had a higher than expected number of deaths from CVA (observed = 72, expected = 61: 10%) than non-diabetic men (observed = 1,240, expected=1250: 9%), with diabetic men having an increased odds ratio (1.2) (*P*=N.S). CVA was the underlying cause of death in 1,907 non-diabetic women and in 79 diabetic women (reducing the estimated relative risk for diabetic women: OR=0.9) ($P < 0.05$).

Four hundred and twenty-four (30%) diabetic people in comparison to 12,559 (46%) non-diabetic people died of CHD (*P* <0.001). However, fewer women with diabetes (n=198: 27%) died from CHD than non-diabetic women (n=5,573, 42%) (*P* <0.001); women with diabetes had an increased estimated relative risk of dying from CHD between the ages of 45-54 years (OR=1.3) and 55-64 years (OR=1.4) but reduced risk from 65 years of age onwards (Table 3.4).

Age Groups (years)	Number of Deaths from CHD in Diabetic Women O	(%)	E	Number of Deaths from CHD in Non-Diabetic Women O	(%)	E	*P* (χ^2)	Odds Ratio	95% C.I. Odds Ratio
>85	19	(14)	33	1161	(34)	1113	<0.001	0.30	0.18, 0.49
75-84	56	(21)	81	2157	(46)	2091	<0.001	0.30	0.22, 0.40
65-74	77	(36)	62	1373	(49)	1346	<0.001	0.57	0.43, 0.76
55-64	41	(47)	20	552	(39)	558	N.S	1.36	0.88, 2.10
45-54	5	(26)	3	101	(21)	102	N.A.	1.29	0.46, 3.68
35-44	0	(0)	N.A.	30	(17)	N.A.	N.A.	N.A.	N.A.
<1-34	0	(0)	N.A	1	(2)	N.A	N.A	N.A.	N.A.
Totals	198			5375					

O: Observed E: Expected %: Percentage of total deaths in each age band N.S.: Not significant
Level of significance: $P \leq 0.05$ N.A.: Not applicable (expected frequency < 5 for χ^2 test) N.D.: Not done
CI: Confidence Interval

Table 3.4: Deaths in women from coronary heart disease

There were no differences in deaths from CHD in men between the groups (diabetic: n=226 [33%] and non-diabetic: n=4150 [30%]) (*P*=N.S.).

Fewer people with diabetes [men: n= 59 (9%) and women: n= 59 (8%)] died from malignant neoplasm as the underlying cause of death than expected when compared to non-diabetic people [men: n=3,638 (26%) and women:

n=3,050 (23%)] (*P* <0.001) (Table 3.5 and 3.6). The estimated relative risk of dying from malignant neoplasm was greatly reduced in both men and women with diabetes (OR=0.3 for both sexes).

Age Group (years)	Deaths from Neoplasms Diabetic Men			Deaths from Neoplasms Non-Diabetic Men			*P* (χ^2)	Odds Ratio	95% C.I. Odds Ratio
	O	(%)	E	O	(%)	E			
>85	5	(13)	6	170	(15)	169	N.S.	0.83	0.32, 2.15
75-84	12	(6)	48	930	(24)	895	<0.001	0.20	0.11, 0.37
65-74	23	(9)	82	1363	(33)	1304	<0.001	0.53	0.35, 0.82
55-64	15	(12)	44	835	(36)	806	<0.001	0.23	0.14, 0.41
45-54	1	(<1)	10	245	(32)	236	<0.001	0.07	0.01, 0.50
35-44	3	(20)	4	60	(28)	59	N.S.	N.D.	N.D
15-34	0	(0)	0	35	(35)	35	N.A	N.D	N.A.
Totals	59			3638					

O: Observed E: Expected %: Percentage of total deaths in each age band N.S.: Not significant
Level of significance: $P \leq 0.05$ N.A.: Not applicable N.D.: Not done C.I.: Confidence Interval

Table 3.5: Deaths in men from malignant neoplasms

Age Group (years)	Deaths from Neoplasms Diabetic Women			Deaths from Neoplasms Non-Diabetic Women			*P* (χ^2)	Odds Ratio	95% C.I. Odds Ratio
	O	(%)	E	O	(%)	E			
>85	3	(2)	15	333	(11)	321	<0.001	0.18	0.06, 0.56
75-84	15	(<1)	51	817	(19)	781	<0.001	0.24	0.14, 0.41
65-74	27	(13)	71	903	(35)	859	<0.001	0.27	0.18, 0.40
55-64	11	(13)	40	626	(49)	597	<0.001	0.15	0.08, 0.28
45-54	3	(16)	11	253	(60)	245	<0.001	0.13	0.04, 0.45
35-44	0	(0)	3	96	(65)	93	N.A.	N.A.	N.A.
15-34	0	(0)	1	22	(40)	21	N.A.	N.A.	N.A.
Totals	59			3050					

O: Observed E: Expected %: Percentage of total deaths in each age band N.S.: Not significant
Level of significance: $P \leq 0.05$ N.A.: Not applicable N.D.: Not done C.I.: Confidence Interval

Table 3.6: Deaths in women from malignant neoplasms

In certain age bands there were fewer deaths from gastro-intestinal disease and "Other" underlying causes in comparison to non-diabetic people in both men and women (Table 3.7).

Causes of Death	Age Bands (years)	Numbers of Deaths Diabetic Group O (%) E	Numbers of Deaths Non-Diabetic Group O (%) E	*P*
G.I. (Men)	75-84	1 (0.5) 7	139 (4.0) 133	<0.05
	65-74	1 (0.4) 7	125 (3.0) 119	<0.05
G.I. (Women)	74-84	3 (1.0) 9	143 (3.0) 137	<0.05
	65-75	0 (0.0) 7	90 (3.0) 83	<0.01
Others (Men)	75-84	2 (1.0) 14	299 (7.0) 287	<0.001
	65-74	0 (0) 12	218 (5.0) 206	<0.001
	55-64	1 (0.8) 7	133 (5.0) 127	<0.05
Others (Women)	>85	1 (0.7) 15	387 (11.0) 373	<0.001
	75-84	3 (1.0) 26	462 (10.0) 439	<0.001
	65-74	2 (0.9) 15	200 (7.0) 190	<0.001

O: Observed E: Expected %: Percentage of total deaths in each age band N.S.: Not significant
Level of significance: $P \leq 0.05$ G.I.: Gastro-Intestinal

Table 3.7: Other underlying causes of death in different age groups

However, there was a higher proportion of deaths from liver disease in diabetic men from the ages 55-64 years (1.5%) compared to 0.5% in non-diabetic men and 65-74 years (diabetic men: 1.5% and non-diabetic men: 0.4%), and in diabetic women from 75-84 years (1.00%) and >85 years (1.00%) compared to 0.01% and 0.03% respectively in non-diabetic women of the same age group. The numbers in diabetic people were very small.

Two hundred and eighty seven (20%) of the diabetic group died from diabetes and there was no significant difference between males and females. Twenty died from "acute diabetes" i.e. DKA, HOC and HG, 50 from

nephropathy, and the rest from diabetes, intercurrent infection or gangrene (Table 3.8).

Causes of Death (Diabetes)	Number of Deaths Male			Number of Deaths Female			*P*
	O	(%)	E	O	(%)	E	
Acute	13	(10)	9	7	(5)	11	N.S.
Nephropathy	20	(15)	23	30	(19)	27	N.S.
Others Diabetes	100	(75)	101	117	(76)	116	N.S.
Total Diabetes	133	(100)	137	154	(100)	149	N.S.

O: Observed E: Expected N.S.: Not significant Level of significance: $P<0.05$

Table 3.8: Deaths from diabetes.

More women (n=96) than men (n=59) died of diabetes from the age of 75 years onwards ($P<0.005$) but there was no significant difference between the sexes under 75 years of age. In the sub-group of acute diabetes there were more male deaths (n=9) between 65-74 years than expected (n=6) ($P<0.05$) and only 2 deaths under the age of 55 years. When nephropathy was compared to other causes within the diabetic group, it was not a significant underlying cause of death. The only notable difference in deaths from nephropathy, was that men died of nephropathy from the age of 35 years onwards, whereas the women were older, 45 years and above.

3.6 Discussion

All studies of mortality that rely on information recorded on death certificates (or in this case "second-hand" information with death certificates as the original source) are potentially subject to inaccuracy secondary to what is actually written as the cause of death in Part 1 of the certificate, which at times, may not reflect the underlying diseases which have contributed to the death (Fuller *et al,* 1983; Panzram, 1987). A study in Minnesota identified that diabetes was not mentioned in 46% of 274 death certificates of people known to have diabetes (Sprafka *et al*, 1993). It is likely that under reporting of diabetes as the underlying cause of death also applied to the data in this study.

Under reporting of diabetes on death certificates can bias the results of a study, especially in diabetic people who have macrovascular complications, including coronary heart disease. The other potential source of bias could be inaccuracy on recording the cause of death in nephropathy patients. This was observed in a study into the availability of RRT in diabetic patients, where it was noted that in some patients congestive cardiac failure was recorded on the death certificate. The authors commented that ESRF would have been more appropriate as the terminal stages of ESRF include the symptoms of cardiac failure (Working Party of the Renal Association *et al*, 1989).

The most common underlying cause of deaths in both diabetic (53%) and non-diabetic groups (47%) was CVD. The proportion of deaths from CVD in diabetic people was lower than in a Dutch study but similar to other studies in this country (de Grauw *et al*, 1994; Gatling *et al*, 1997). The higher percentage of deaths from CVD in both male groups can be compared with a study in Sweden, where it was shown that 11.6% of diabetic and 2.7% of non-diabetic men died from CVD (Rosengren *et al*, 1989). This can be interpreted as indicating that death rates for CVD in people with diabetes in Wolverhampton are exceptionally high. This comparison also shows that more diabetic men die from CVD than non-diabetic men, similar to the present study. The Wolverhampton results are comparable with those of a study in Minnesota, USA, which looked specifically at mortality in Type 2 diabetic patients, where CVD accounted for 63% of deaths in men and 62% on women (Sprafka *et al*, 1993).

It has been demonstrated previously that people with diabetes have an increased risk of death from CHD (Kannel and McGee, 1979; Waine, 1989). The present study has shown that CHD was the major cause of death in both diabetic and non-diabetic groups in Wolverhampton. There was no difference in deaths from CHD between the male diabetic and non-diabetic groups while the smaller proportion of deaths from CHD in diabetic women was in contrast to a study in Aberdeen where diabetic women had a higher than expected death rate from CHD than non-diabetic women (Wong *et al*, 1991). It has been reported that diabetic women are six times more likely to develop CHD than non-diabetic women (Manson *et al*, 1991). In

Wolverhampton diabetic women between the ages of 45 and 64 years had an increased estimated relative risk of dying from CHD than non-diabetic women, but this estimated risk was far lower than the risk of developing CHD that was suggested by Manson and co-workers (1991). A report published in 1994 stated that Wolverhampton had one of the highest death rates from CHD in the UK (Kelleher *et al*, 1994). Extrapolating that this may be typical of mortality in the town over several years, it may (a) explain the lower proportion of deaths with CHD as the underlying cause of death in diabetic women found in the present study in comparison to studies elsewhere and (b) complicate the comparison of deaths in Wolverhampton with studies from other places which have a lower population mortality rate from CHD. CVA represented a small proportion of cardiovascular deaths in both groups (similar to the findings of Wong and colleagues, 1991).

The small proportion of deaths from malignant neoplasms in both male (9%) and female (8%) diabetic patients were consistent with the findings of Fuller and co-workers (1983). The Minnesota study demonstrated that deaths from neoplasms occurred in almost equal percentages in male and female diabetic patients; this was consistent with the Wolverhampton study (Sprafka *et al*, 1993). Few other studies have looked at mortality from liver and gastro-intestinal diseases. The small proportion of deaths from gastro-intestinal disease (9%) in diabetic patients was interesting but was also consistent with the findings in a study by Kessler published in 1971 of over 10,000 diabetic deaths, where the numbers of deaths from liver and gastrointestinal disease were very small and those from cancer accounted

for only 9% of deaths (Kessler, 1971). It is possible that the reduced mortality from both malignant neoplasm and gastro-intestinal disease is due to "competitive risk" i.e.: one predominant cause of mortality e.g.: CVD, in a group of people results in reduced mortality from other causes (Fuller *et al*, 1983). The higher proportion of deaths from liver disease (18%) in diabetic people in comparison to non-diabetic people (<1%) may be secondary to a number of factors e.g.: in people with diabetes, the metabolic functions of the liver are abnormal in terms of gluconeogenesis, insulin metabolism and lipid metabolism (Type 2) which may eventually result in liver damage. Diabetic patients may also be taking a number of drugs for diabetes and the complications of the disease, which potentially could have damaging effects on the liver over time. However, the numbers of deaths from liver disease in each of the groups was relatively small. Further investigation of the role of liver disease in diabetic deaths may be an interesting area for future research.

Mortality directly attributable to diabetes represented a fifth of all diabetic deaths. Only a small number of these deaths were due to the acute metabolic complications of diabetes with 10% of the "acute" diabetic deaths occurring under the age of 50 years. This contrasts to the findings of Turnbridge's group in 1981, who found that 19% of diabetic deaths under 50 years were due to diabetic ketoacidosis and hypoglycaemia. More recently, Tattersall and Gill outlined the difficulties in establishing the cause of sudden death in diabetic patients which included how unreliable measurement of post-mortem blood glucose can be and the reluctance of

pathologists to attribute cause of death to metabolic rather than pathological causes (Tattersall and Gill, 1991). The data presented here should be assessed in this light.

In relation to other causes of death in this study, diabetic nephropathy was not a significant underlying cause of death. However, as previously discussed, cardiovascular disease may be recorded as the underlying cause of death in patients in ESRF either secondary to renal failure or because many patients in ESRF die of ischaemic heart disease (Joint Working Party of the Renal Association *et al*, 1989; Wong *et al*, 1991). The Aberdeen study found that 32% of deaths in patients with nephropathy were due to ischaemic heart disease which was not surprising as people with nephropathy often have accompanying macrovascular complications (Wong *et al*, 1991). It has also been suggested that mortality rates from nephropathy in people below 45 years of age may be reduced due to the increasing numbers of diabetic patients receiving renal replacement therapy (Stephenson *et al*, 1992). However, Moss and colleagues in Wisconsin, found excess mortality in both younger and older diabetic patients due to renal disease and have suggested that if nephropathy had been classified under diabetes using ICD-09 coding, the excess mortality would in fact be greater than they found (Moss et al, 1991). More recently, excess mortality associated with proteinuria and accompanying hypertension in both insulin dependent and non-insulin dependent diabetic patients has been shown on a world wide scale (Wang *et al* , 1996).

Despite reservations about the reliability of data from death certificates, this section of the thesis provided an interesting background to the causes of death in diabetic patients in Wolverhampton and specifically as to whether diabetic nephropathy was significant in terms of mortality. However, there were a number of flaws with the design of this study. Firstly, the data used on deaths in the diabetic group were not obtained directly from death certificates but through a "mediator", the Department of Public Health, which produced two specific problems: There was no guarantee that data on all deaths in diabetic patients had been obtained and when comparison was made with the OPCS data it became evident that a proportion of deaths in diabetic people had been omitted from the data source provided by the Department of Public Health. This was not entirely surprising as this data had been provided to inform the diabetic physicians about deaths in their patient population and not specifically for research purposes. The other problem with this data was that the only method of validating the accuracy of the information was to compare some of the data with death certificates. Unfortunately, the Registrar of Births, Deaths and Marriages would only agree to this if copies of the death certificates were purchased at £5.00 each; to validate 5% of this data would have cost over £350 and there was no funding available for this project.

During the years 1981-1986, there was no information on the total population attending the diabetic clinic. This limited the types of analysis which could be performed and essentially excluded performing annual and standardised mortality rates in the diabetic group although these could have

been calculated in the general population (Gordis, 1996). As the data did not include information on deaths of all people with diabetes calculation of proportionate mortality rates (PMR) would have been inaccurate. However, this could have been calculated for the sample of diabetic deaths, out of interest, but a direct comparison of the PMR in the general population would not have been valid due to the missing data (Gordis, 1996).

Although a comparison of causes of death in different age groups has been made, again due to the incomplete data on deaths in diabetic people and the lack of information on the total diabetic population, it was impossible to calculate age-specific or age-standardised death rates (Gordis, 1996).

The present study was retrospective using available data gathered for other purposes. In order to obtain more robust data, a prospective cohort study over ten years would have allowed for efficient project management, development of a sound scientific protocol, removal of possible biases, collection of data from a well documented group of diabetic patients (and the general population if wished) and the potential to analyse the data in a more comprehensive manner. This type of study was not possible in the lifetime of this thesis.

3.7 Conclusions

1 The majority of deaths in both diabetic and non-diabetic people in Wolverhampton were due to CVD with CHD being the main underlying cause of death.

2 Female diabetic patients had a 4.0 times increased risk of dying from CVD than non-diabetic females between the ages of 45-54 years and a 2.5 times increased risk between ages of 55-64 years in comparison to diabetic men where the risk was 2.4 times increased at ages 45-54 years and 1.6 times increased between 55-64 years. Therefore accept $H_o(2)$: There was no difference in underlying causes of death between men and women with diabetes; but with the caution that diabetic women had an increased risk of developing CVD.

3 Diabetic patients were less likely to die from malignant neoplasms, gastro-intestinal disease and other causes than non-diabetics. Therefore accept $H_1(1)$: There was a difference in underlying causes of death in people with and without diabetes.

4 Diabetes was responsible for 20% of deaths within the diabetic group.

5 Diabetic nephropathy was not a significant cause of death. Therefore, accept H_o(4):Diabetic nephropathy was not a major underlying cause of death in people with diabetes.

The results obtained neither proved or disproved the third hypothesis: That there was no difference in underlying causes of death in people with diabetes of different ages.

Chapter 4

Renal Disease in Patients Attending the Wolverhampton Diabetic Clinic: 1987-1995

4.1 Introduction

The aim of the clinician is to provide diabetic care, education and support to ensure that patients manage their diabetes in a way that will prevent the occurrence of severe complications. Diabetic nephropathy is one of those complications which becomes devastating when patients reach end stage renal failure and the only alternatives are dialysis, kidney transplantation or death.

Several large studies; population based, clinic based and across Europe, have been undertaken to determine the extent of nephropathy within a specified population; comparisons have also been made between the USA and Europe (Klein *et al*, 1988; Humphrey *et al*, 1989; Stephenson *et al*, 1994; Lloyd *et al*, 1996). However, from a clinical perspective it is more interesting to know how many local diabetic patients have nephropathy in order to provide adequate provision of care for these patients.

In 1988-1989, the Wolverhampton hospital diabetic clinic served a population of 289,200 plus approximately 31,000 diabetic people from surrounding areas (South and Mid-Staffordshire, Shropshire, Dudley and Walsall) (Office of Population Censuses and Surveys, 1990). The number of patients attending the clinic between 1988-1989 was 2,047 from a total diabetic population in the town of 5,155 (Wolverhampton Diabetes Register, 1990). The extent of renal disease and particularly diabetic nephropathy in the clinic was not known although

increasing numbers of patients were being referred from the diabetic clinic for nephrological assessment. The aim of this study was to establish the extent of diabetic nephropathy in patients attending the hospital clinic.

4.2 The Objectives

1 To determine the prevalence of nephropathy in the index diabetic population.

2 To determine the prevalence of other causes of renal disease in the index diabetic patients.

3 To determine the prevalence of other diabetic complications in the index patients.

4.3 Hypotheses

1 H_0: The majority of patients being referred for nephrological assessment do not have diabetic nephropathy.

H_1: The majority of patients being referred for nephrological assessment have diabetic nephropathy.

2 H_0: There was no difference in prevalence of diabetic complications between Type 1 and Type 2 patients with renal disease.

H_1: There was a difference in prevalence of diabetic complications between Type 1 and Type 2 patients with renal disease.

4.4 Methods

Data on the numbers of patients (n=2047) attending the diabetic clinic in 1988-1989, obtained from the Wolverhampton Diabetic Clinic computerised register, were used to determine the prevalence of patients with renal disease in the clinic population, as data for subsequent years was not available. Data were collected from the hospital records of all diabetic patients referred from the diabetic clinic for nephrological assessment over an eight-year period between 1987-1995. This included patients with proteinuria and raised serum creatinine levels, with or without proteinuria, and patients presenting with end stage renal failure who had defaulted from routine clinic visits. Data on age, sex, ethnic group, family history of diabetes, smoking history, diabetes, diabetes treatment and diabetic control (as measured by HbA1), and complications including renal disease (as measured by 24 hour urinary protein and serum creatinine concentrations) were collected. Details of regular defaulting from routine diabetic clinic visits (i.e.: missing more than two consecutive clinic visits in more than one year since diagnosis of diabetes) were also collected. Patients were divided into Type 1 and Type 2 diabetes groups and a comparison was made between the two. Type 1 was defined as diagnosis before the age of 35 and requiring insulin treatment; Type 2 was defined as diagnosis after 35 years of age and treated with either diet, oral therapy or insulin treatment. Normal blood pressure was defined as a systolic pressure of $\leq$ 140 mm Hg and a diastolic $\leq$ 90 mm Hg as recommended by the Working Group on Hypertension in Diabetes (1987).

Diabetic nephropathy was diagnosed on clinical grounds: the presence of proteinuria and diabetic retinopathy. Renal biopsy was only performed where there was insufficient clinical evidence to give a definitive diagnosis. For patients with proteinuria at diagnosis of diabetes the duration of diabetes was recorded as zero, only to give a numerical value, as it was accepted that Type 2 patients presenting with complications will have had diabetes for some time before diagnosis.

Diabetic retinopathy, peripheral neuropathy and peripheral vascular disease were graded according to severity, as recorded in patients notes. Ischaemic heart disease was assessed by either documented evidence of angina symptoms or on electrocardiographic evidence.

Descriptive statistics, two-tailed Student t tests, Chi^2 tests (Fisher Exact test was used if $n \leq 5$) and Mann-Whitney U tests for non-parametric data were performed using SPSS and Excel statistics packages. Level of statistical significance was $P \leq 0.05$.

4.5 Results

All percentages relate to patients referred for nephrological assessment (index group) unless otherwise stated. Over eight years, 220 people (11% of the hospital diabetic population) were referred for nephrological assessment, of these 49 (22%) were Type 1 and 171 (78%) were Type 2 diabetic patients. All Type 1 and 84 (49%) Type 2 patients were treated with insulin (it was inapplicable to perform statistical tests to compare differences in treatment). Seventy-seven (35%) were female and 143 (65%) were male (Table 4.1). There were 153 (70%) White, 44 (20%) Indo-Asians and 23 (10%) Black. Two Type 1 Indo-Asian patients (33% of all Type 1 Indo-Asians) and 21 Type 2 Indo-Asian patients (55% of all Type 2 Indo-Asians) spoke little or no English.

Characteristic	Type 1 n (%)	Type 2 n (%)	*P*
TOTAL	49 (22)	171 (78)	
GENDER			
Male	35 (71)	108 (63)	N.S.
Female	14 (29)	63 (37)	
ETHNIC GROUP			
White	40 (82)	113 (66)	
Indo-Asian	6 (12)	38 (22)	<0.005
Black	3 (6)	20 (12)	
TREATMENT			
Insulin	9 (100)	84 (49)	
Oral	0	75 (44)	N/A
Diet	0	12 (7)	

N.S.: Not Significant N/A: Not Applicable Level of significance: $P \leq 0.05$

Table 4.1: Demographics of diabetic patients with renal disease.

The age range in both groups was wide, with a mean age of 43 (SD ±12) years in Type 1 patients compared to 63 (SD ±8) years in the Type 2 group; this was significantly different (P<0.001). The mean duration of diabetes at referral was 23 (SD ±10) years in the Type 1 group and 11 (SD ±7) years for Type 2 patients (P<0.001) and the mean duration of diabetes at the onset of proteinuria was longer in Type 1 patients: 19 years (SD ±8) years versus Type 2: 11 (SD ±7) years (P<0.001).

Renal biopsies were performed in 21 cases where there was doubt as to the diagnosis on clinical grounds alone, only three were in Type 1 patients. Diabetic nephropathy was diagnosed in 47 (96%) of Type 1 patients and 102 (60%) of the Type 2 group. Chronic renal failure secondary to hypertension was diagnosed in 38 (22%) of Type 2 patients while 31 had renal disease due to other causes, including four with confirmed renal artery stenosis (Figure 4.1). Four (2%) Type 2 patients had proteinuria at diagnosis of diabetes.

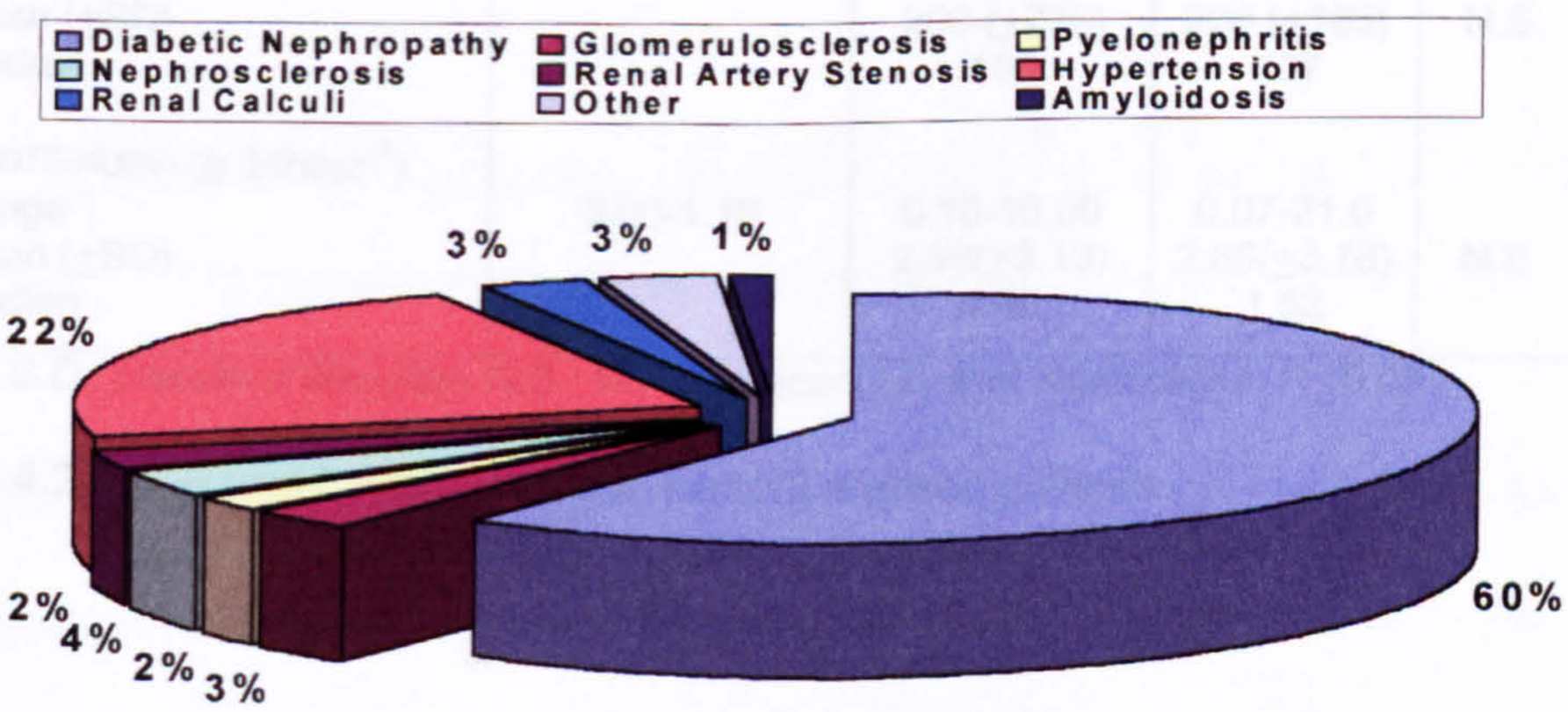


Figure 4.1: Causes of renal disease in Type 2 diabetic patients

The power of the study was calculated as 99% using the proportion of patients in each group with a diagnosis of diabetic nephropathy (0.96 in Type 1 patients and 0.60 in Type 2 patients), $P \leq 0.05$ and the harmonic mean of the number in each group (as the sample sizes were unequal in the two groups).

4.5.1. Renal Function

The mean urinary protein excretion over 24 hours was the same in both groups although the range was greater in Type 2 patients. There was a wide range of serum creatinine levels with a bimodal distribution in both groups; however, the means were similar although the standard deviation in the Type 2 group was smaller and the median for Type 1 patients was within the normal limit (Table 4.2).

	Normal Range	Type 1	Type 2	*P*
SERUM CREATININE (μmol l^{-1})				
Range	60-120	40-1070	57-1140	
Mean (±SD)		209 (±235)	206 (±185)	N.S.
Median		105	137	
PROTEINURIA (g 24hour^{-1})				
Range	0.00-1.10	0.10-16.00	0.07-21.0	
Mean (±SD)		2.98(±3.13)	2.86(±3.78)	N.S.
Median		1.89	1.52	

S.D.: Standard Deviation N.S.: Not Significant Level of significance: $P \leq 0.05$

Table 4.2: Renal function in Type 1 and 2 diabetic patients

4.5.2 *Microvascular Complications*

Of the 146 (66%) patients with retinopathy, 90 (62% of retinopathy patients) had severe retinopathy. There was a higher prevalence of retinopathy in the Type 1 group (92%) than in Type 2 patients (59%) ($P<0.001$); Type 1 patients had significantly more severe retinopathy ($P<0.05$). Forty-nine percent of patients with retinopathy were treated by laser photocoagulation. Fifty patients (23%) had cataracts, 23 (10%) were blind in one eye and 10 (4%) were blind in both eyes; Type 1 patients had a higher prevalence of blindness (Table 4.3).

	Type 1 n (%)	Type 2 n (%)	*P*
EYES			
Retinopathy	45 (92)	101 (59)	<0.001
Severe Retinopathy	39 (87*)	51 (51*)	<0.05
Laser Photocoagulation	29 (64*)	43 (43*)	N.S.
Cataracts	14 (29)	36 (21)	N.S.
Blind One Eye	13 (27)	10 (6)	<0.001
Blind Both Eyes	5 (10)	4 (2)	<0.005
NERVOUS SYSTEM			
Peripheral Neuropathy	23 (47)	57 (33)	N.S.
Autonomic Neuropathy	4 (8)	3 (2)	<0.01
KIDNEYS			
Diabetic Nephropathy	47 (96)	10 (60)	<0.001

*: % of patients with retinopathy N.S.: Not significant Level of significance: $P \leq 0.05$

Table 4.3: Microvascular complications in diabetic patients with renal disease

Peripheral neuropathy was identified in 80 (36%) patients, in seven this was severe, while autonomic neuropathy was found in seven (3%) patients. The Type 1 group had a higher prevalence of peripheral neuropathy (N.S.) and autonomic neuropathy ($P<0.01$) (Table 4.3).

4.5.2. Macrovascular Complications

Ischaemic heart disease (IHD) was found in 41 (19%) patients and 26 (12%) had had myocardial infarctions (MI). The Type 1 group had a slightly lower prevalence of IHD and MI than Type 2 patients had but this was not statistically significant. Transient ischaemic attacks (TIA) had occurred in 8 (4%) patients and cerebrovascular accidents (CVA) in 22 (10%). The Type I patients had a non-significant higher prevalence of CVA (Table 4.4). Total cholesterol levels (Type 1: mean: 6.8 (S.D.$\pm$ 2.0); Type 2: mean: 6.6 (S.D. $\pm$ 2.0) mmol/l) and diabetic control (Type 1: mean HbA1 11.5 (S.D.$\pm$ 2.2) %; Type 2: mean 10.9 (S.D.$\pm$ 2.5) %) were similar in both groups (*P*=N.S. for both). Peripheral vascular disease (PVD) was present in 68 (31%) patients and was severe in 24 of these. Twenty-five patients (11%) had had leg or foot ulcers and 9 (4%) had one leg amputated.

	Type 1 n (%)	Type 2 n (%)	*P*
HEART			
Ischaemic Heart Disease	8 (16)	33 (19)	N.S.
Myocardial Infarction	4 (8)	22 (13)	N.S.
BRAIN			
Transient Ischaemic Attack	2 (4)	6 (4)	N.S.
Cerebrovascular Accident	6 (12)	16 (9)	N.S.
VASCULAR			
Peripheral Vascular Disease	19 (39)	49 (29)	N.S.
Ulcers	5 (10)	20 (12)	N.S.
Leg Amputations (1 only)	3 (6)	6 (4)	N.S.

N.S.: Not significant Level of significance: $P \leq 0.05$

Table 4.4: Macrovascular complications in diabetic patients with renal disease

There was no statistically significant difference between the groups for any of these complications (Table 4.4).

Hypertension was present in 202 (92%) patients; the prevalence in both groups was similar. Only 95 patients with hypertension were being treated with anti-hypertensive medication at referral (Table 4.5). The mean and median systolic blood pressures, which were higher in Type 2 patients, demonstrated that systolic hypertension was common in both groups. The mean and median diastolic pressures for both groups were 90mmHg. Of those receiving anti-hypertension medication, around a half had hypertension that was resistant to treatment with one drug only; these patients.were treated with a combination of drugs.

	Type 1 n (%)	Type 2 n (%)	*P*
Hypertension	44(90)	158(92)	N.S.
Anti-hypertensive Therapy	20(46*)	75(47*)	N.S.
Systolic Pressure (mm Hg)			
Range	70-240	110-260	
Mean (±SD)	160 (±30)	168 (±26)	N.S.
Median	160	163	
Diastolic Pressure (mm Hg)			
Range	40-120	50-120	
Mean (± SD)	90 (±15)	90 (±14)	N.S.
Median	90	90	

S.D.: Standard deviation N.S.: Not significant Level of significance: $P \leq 0.05$

*: % of patients with hypertension

Table 4.5: Blood pressure of diabetic patients with renal disease

Sixty-two patients smoked cigarettes, 76 had a family history of diabetes and 75 had defaulted from routine diabetic clinic follow up. There were no significant differences between the groups for any of these factors.

4.5.4. Outcomes

Over eight years, 82 (37%) patients reached end stage renal failure (ESRF) or died. Of those who received renal replacement therapy (RRT): 16 had Type 1 and 26 had Type 2 diabetes. Of the Type 1 patients on RRT, 11 received continuous ambulatory peritoneal dialysis (CAPD), five had haemodialysis (HD) and seven eventually received kidney transplants. In comparison, 13 Type 2 patients received CAPD, 13 HD and only two had kidney transplants; one went to India to be transplanted (Figure 4.2).

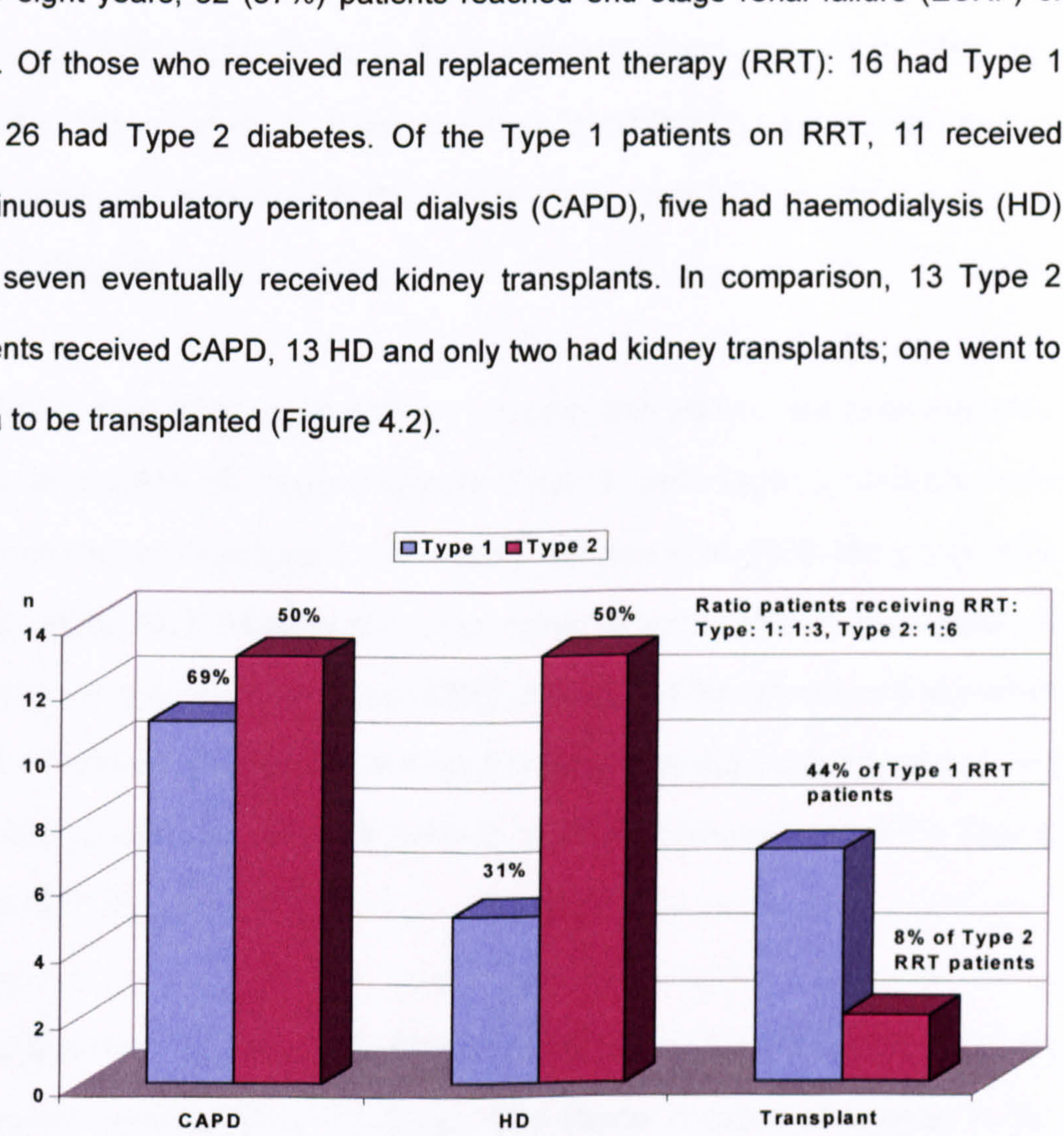


Figure 4.2: Type of renal replacement therapy received by diabetic patients

Of the 40 patients who died, 15 (38%) died from ESRF either following or without having received RRT, 17 (42%) from cardiovascular disease and eight (20%) from other causes.

4.6 Discussion

Seven percent of the diabetic clinic population had nephropathy and 4% had renal disease due to other causes. These results can be compared with two studies in Type 1 patients; the EURODIAB study where prevalence was 12% and the Epidemiology of Diabetes Complications study where prevalence was 27% (Lloyd *et al*, 1996). The large numbers of Type 2 patients with nephropathy in this study, 102 (60%), confirms the findings of Humphrey and co-workers (1989). In a population-based study in Minnesota, they found that Type 2 patients had an eight times greater chance of developing chronic renal failure than people with Type 1 diabetes.

The higher prevalence of renal disease in men than women was to be expected. Previous studies of nephropathy in Type 1 and Type 2 patients have demonstrated a preponderance of males (Andersen *et al*, 1983; Humphrey *et al*, 1989). In a study detailing the progression of proteinuria in both types of diabetes, Cooper and colleagues (1988) showed that the ratio of men to women in Type 1 patients was 2:1 and in Type 2 was 1.5:1. In this study the ratio of men to women was higher in Type 1 patients: 2.5:1 and marginally so in the Type 2 group: 1.7:1.

The difference in age at referral for nephrological assessment can be explained by the later onset of Type 2 diabetes. The shorter duration of diabetes in the

Type 2 group both at referral and onset of proteinuria in comparison to Type 1 patients was reflective of the unknown duration of diabetes prior to diagnosis (the silent phase pre-diagnosis). Many Type 2 patients are unaware of the symptoms of diabetes and that by the time the disease is diagnosed, after several years of untreated hyperglycaemia, complications, including nephropathy, have developed. In this study, the 2% of Type 2 patients with proteinuria at diagnosis of diabetes supports this argument and is consistent with the findings of the United Kingdom Prospective Diabetes Study, where they also found 2% of newly diagnosed Type 2 patients to have microvascular complications (UK Prospective Diabetes Study Group, 1994). In 1991, Friedman and Gross demonstrated that the decline in glomerular filtration rate in Type 2 patients is at a slower rate than in Type 1 patients.

Type 1 patients had more microvascular complications than the Type 2 group but this was to be expected, as there was a higher prevalence of nephropathy in the Type 1 group. It has been suggested that a sub-group of diabetic patients are susceptible to both nephropathy and retinopathy (Agardh *et al*, 1989). The EURODIAB study clearly demonstrated the association between retinopathy, nephropathy and raised diastolic blood pressure in a subgroup of Type 1 patients (Stephenson *et al*, 1995). The higher incidence of blindness in Type 1 patients in this study was consistent with the findings of Parving and colleagues (1988), where the prevalence of proliferative retinopathy increased by 36% and blindness by 11% in patients with proteinuria.

The prevalence of peripheral neuropathy in diabetic patients without proteinuria has been shown to be 11% in Type 1 patients and 26% in Type 2 patients (Boulton *et al*, 1985; Franklin *et al*, 1990). It has also been demonstrated that insulin-dependent patients with proteinuria have an increased risk of peripheral neuropathy (Parving *et al*, 1988). This study has shown that Type 2 patients had a relatively high prevalence of peripheral neuropathy (33%) and that there was no significant difference in this complication between the two groups. Peripheral neuropathy, like the other microvascular complications, is the result of poor diabetic control over many years. In this study, HbA1 results at referral were higher than ideal in both the groups.

The prevalence of macrovascular complications were the same in both groups which was surprising considering the younger age of the Type 1 patients. It has been suggested that Type 1 patients with proteinuria have an eight times increased incidence of myocardial infarctions and ischaemic heart disease (Deckert *et al*, 1992). It has been postulated that the mechanisms involved in reducing the density of heparan sulphate proteoglycan which occur in the glomerular basement membrane of patients with proteinuria may also occur in plasma membranes and myomedial cells and thus contribute to atherosclerosis and macrovascular disease (Deckert *et al*, 1992). The relatively high prevalence of cerebrovascular accidents and peripheral vascular disease in Type 1 patients also reflects the high risk of atherosclerosis in these patients.

Hypertension is twice as common in diabetic as in non-diabetic individuals (Working Group on Hypertension in Diabetes, 1987). Rising blood pressure is known to contribute to the progression of diabetic nephropathy (Microalbuminuria Collaborative Study Group, United Kingdom, 1993). The importance of effective blood pressure control in preventing the decline of renal function in diabetic patients was first observed in the early 1980s and various strategies have been used to keep blood pressure within normal limits and decelerate the progression of renal disease (Mogensen, 1982; Parving *et al*, 1983; Hommel *et al*, 1986; Lewis *et al*, 1993; Mogensen, 1995; EUCLID Study Group, 1997). In this study blood pressure levels were similar in both Type 1 and Type 2 patients. Both groups had systolic hypertension and diastolic pressures were at the upper limits of normal. Systolic hypertension has been associated with a decline in renal function in both types of diabetes and diastolic hypertension (≥ 95mmHg) with the progression of nephropathy in Type 1 patients (Cooper *et al*, 1988; Klein *et al*, 1988; Nielsen *et al*, 1993, Deckert *et al*, 1991). Less than half of both groups who were hypertensive were receiving anti-hypertensive medication at referral, which was surprising as over 90% of patients had hypertension. Of those on treatment, around a half had hypertension that was resistant to treatment with one drug only and were receiving a combined adjunct therapy. As renal function deteriorates the blood pressure tends to rise correspondingly and becomes more difficult to control. The need for combined adjunct anti-hypertensive regimes may be indicative of the advanced stage of renal disease of many of these patients.

Renal function varied from within normal levels for serum creatinine to end stage renal failure in both groups and there was no difference between the groups in this respect.

The relatively high default rates from routine diabetic clinic attendance support previous research performed in Wolverhampton which demonstrated that patients defaulting from regular clinic visits had a higher complication rate than regular attendees (Hammersley *et al*, 1985).

This was a prospective study of a cohort of patients with nephropathy which included patients with already established renal disease. This meant that the number of new cases / population/ year (the incidence) could not be calculated but the extent of renal disease in the diabetic clinic could be determined using point prevalence. Point prevalence was used as opposed to period prevalence because diabetic nephropathy is a chronic disease state during which there are rarely periods of remission (Silman, 1995).

In Type 2 patients there has been much debate about using clinical definitions to diagnose nephropathy instead of using renal biopsies to confirm the diagnosis. Without having performed renal biopsy, there is a potential source of biasing the diagnosis in Type 2 patients although previous research has indicated that the diagnosis of nephropathy on histological examination is not as clear-cut as in Type 1 patients (Ritz *et al*, 1995).

Observer bias was probably present in the examination of physical parameters by different clinicians as no specific examination protocol was followed and no training was performed prior to the study to reduce individual variation in techniques. Using data obtained at routine clinic visits without using a specific protocol provided an insight into the real practice of clinical medicine in a way that the use of a formal examination and treatment protocol designed for research purposes would not have given. It could be argued that the results of this project are more relevant to evidence based practice than those from a formal research protocol.

Serum creatinine concentrations were used to assess renal function instead of creatinine clearance; this being a more accurate assessment of renal function that serum creatinine concentration. This was for pragmatic reasons as creatinine clearance was not assessed in all patients but serum creatinine was. In a study based on a structured research protocol as opposed to one based on normal clinical practice, measurements of (a) urinary albumin excretion (b) albumin:creatinine ratio (c) glomerular filtration rate by excretion of radio isotopes and (d) renal plasma flow could have been used to give a more accurate assessment of renal function than serum creatinine levels.

4.7 Conclusions

1 Diabetic nephropathy was the major cause of renal disease in both Type 1 and Type 2 patients, therefore accept $H_1(1)$: the majority of patients referred for nephrological assessment have diabetic nephropathy. However, a significant minority of patients with Type 2 diabetes have either renal failure due to hypertension or to other causes.

2 Type 1 patients in this index population had a higher prevalence of microvascular complications, therefore, accept $H_1(2)$: there was a difference in prevalence of diabetic complications between Type 1 and Type 2 patients.

3 There was no difference in renal function between the two groups.

4 Uncontrolled hypertension was common in both groups.

Chapter 5

Comparison of (i) Type 1 and Type 2 Diabetic Patients with Nephropathy and (ii) Type 2 Patients with Nephropathy and Those with Non-Diabetic Renal Disease

5.1 Introduction

The previous chapter compared the point prevalence of diabetic complications and the pattern of renal disease in Type 1 and Type 2 patients with all causes of renal disease. Whether there are significant differences between (a) Type 1 and 2 patients with nephropathy and (b) between Type 2 patients with nephropathy and those with non-diabetic renal disease needs further elucidation.

There have been many studies looking at the natural history of nephropathy in Type 1 patients (Parving *et al*, 1988; Mathiesen *et al*, 1995). Until it became evident that Type 2 patients formed the majority of people with diabetes and nephropathy receiving renal replacement therapy (RRT), less research had been done to identify the course of nephropathy in this group of patients (Humphrey *et al*, 1989).

A German study has shown that 21 % of Type 2 diabetic patients at autopsy had renal disease from causes other than nephropathy in comparison to dialysis populations where 32% of Type 2 patients in London, and 52% in France had non diabetic renal disease (Grenfell *et al*, 1988; Waldherr *et al*, 1992; Zmirou *et al*, 1992). Within the financial, ethical and time constraints of providing clinical care, it is not always feasible or possible to perform renal biopsies on all Type 2 patients with renal disease to provide a definitive diagnosis. However, by direct comparison of the diabetes and renal histories of Type 1 and Type 2 patients with nephropathy and non-diabetic renal

disease, it is possible to identify characteristics in each group that are predictive of the diagnosis without renal biopsy.

The aim of this part of the study was to determine differences in renal and diabetes parameters between a) Type 1 and Type 2 patients with nephropathy and b) Type 2 patients with nephropathy and patients with non-diabetic renal disease, and c) to establish the magnitude of those differences.

5.2 Objectives

1(a) To ascertain whether there were significant differences in renal function between Type 1 and Type 2 patients with nephropathy.

1(b) To ascertain whether there were any significant differences in the prevalence of diabetic complications between Type 1 and Type 2 patients with nephropathy.

1(c) To identify factors that might be predictive of the development of nephropathy in the two groups.

1(d) To determine whether there were differences in renal outcomes between Type 1 and Type 2 patients with nephropathy.

2(a) To determine whether there were significant differences in renal function between Type 2 patients with nephropathy and non-diabetic renal disease.

2(b) To determine if there were significant differences in prevalence of diabetic complications between Type 2 patients with nephropathy and non-diabetic renal disease.

2(c) To determine whether there were differences in renal outcomes between Type 2 patients with nephropathy and non-diabetic renal disease.

5.3 Hypotheses

1 H_0: The rate of progression of nephropathy was the same in Type 1 patients and in Type 2 patients.

H_1: The rate of progression of nephropathy is different in Type 1 patients to that in Type 2 patients.

2 H_0: Diabetic complications were similar in Type 1 and Type 2 patients with nephropathy

H_1: Diabetic complications were different in Type 1 and Type 2 patients with nephropathy.

3 H_0: Diabetic complications were similar in Type 2 patients with nephropathy compared to patients with non-diabetic renal disease.

H_1: Diabetic complications were different in Type 2 patients with nephropathy compared to patients with non-diabetic renal disease.

4 H_0: The progress of renal disease in patients with nephropathy was the same as that of patients with non-diabetic renal disease.

H_1: The progress of renal disease in patients with nephropathy was faster than that of patients with non-diabetic renal disease.

H_2: The progress of renal disease in patients with nephropathy was slower than that of patients with non-diabetic renal disease.

5.4 Methods

Data collected from the hospital records of patients referred for nephrological assessment (as in Chapter 4: information on diabetes, diabetes history, treatment and complications and renal function) were used to investigate differences between Type 1 and Type 2 patients with nephropathy and Type 2 patients with nephropathy and Type 2 patients with non-diabetic renal disease. Point prevalence was determined for diabetic complications at the nephrological assessment visit.

Duration of diabetes to the onset of proteinuria, retinopathy and hypertension was also determined and compared in a subgroup of patients with these three complications. The subgroup was composed of patients with all three complications, those without either retinopathy or hypertension were omitted. Only patients with a diagnosis of diabetes greater than three years were included, in order to exclude patients with established retinopathy and proteinuria before diagnosis of diabetes and to reduce the possible "unknown" duration of diabetes before diagnosis.

Renal function was assessed using revised results for serum creatinine and 24 hour urinary protein excretion. The revised results were those with "outliers" removed. The "outliers" for creatinine results represented patients with serum creatinine greater than 300 $\mu mol\ l^{-1}$ and for proteinuria those with protein excretion greater than 10g $24h^{-1}$. Data are presented comparing results for all patients, revised and "outliers".

Comparisons were made between (a) Type 1 and Type 2 patients with nephropathy and (b) Type 2 patients with nephropathy and Type 2 patients with non-diabetic renal disease.

Descriptive statistics, two-tailed Student t tests, Chi^2 tests, Mann-Whitney U tests for non-parametric data, the Pearson test for correlation (a maximum of two outlying points were removed per test to make correlation more accurate, when necessary), linear regression and stepwise multiple regression analysis were performed using SPSS and Excel statistics packages. The chosen level of statistical significance was $P \leq 0.05$.

5.5 Results

5.5.1 Comparison of Type 1 and Type 2 Patients with Nephropathy

There were 149 patients with nephropathy, 47 Type 1 and 102 Type 2. Renal biopsies were performed in two Type 1 patients and seven of the Type 2 group to confirm diagnosis in those patients where clinical findings were inconclusive. The ratio of males to females was just over 2:1 with the Type 1 group being predominantly White (83%) in comparison to only 57% White patients in the Type 2 group. Sixty eight (67%) of Type 2 patients were insulin treated (Table 5.1). Diabetic control was poor at referral in both groups.

Characteristic	Type 1 n (%)	Type 2 n (%)	*P*
TOTAL NUMBER	47 (100)	102 (100)	
GENDER			
Male	33 (70)	70 (69)	N.S.
Female	14 (30)	32 (31)	
ETHNIC GROUP			
White	39 (83)	58 (57)	
Indo-Asian	5 (11)	30 (29)	<0.01
Black	3 (6)	14 (14)	
TREATMENT			
Insulin	47 (100)	68 (67)	
Oral	0	32 (31)	N.A
Diet	0	2 (2)	
DIABETIC CONTROL			
HbA1 (%)			
Range	7.4-16.3	6.7-20.0	
Mean (±SD)	11.9 (2.1)	11.2 (2.5)	N.S.

N.S.: Not significant N.A.: Not applicable Level of significance $P \leq 0.05$

Table 5.1: Demography of Type 1 versus Type 2 patients with diabetic nephropathy

The age range at referral was wide with a twenty year difference in mean ages: Type 1: range: 18-71, mean: 43 (SD±12) years and Type 2: range: 41-83, mean: 62 (SD±8) years (P<0.001). The mean duration of diabetes was twelve years shorter in the Type 2 group than in Type 1 patients and this was statistically significant: Type 1: 24 (± 8) years versus Type 2: 12 (±7) years (P<0.001).

In each of the two sub-groups of patients with nephropathy and retinopathy (n= 36, Type 1 and n= 40, Type 2) the mean duration of diabetes before onset of proteinuria was shorter in the Type 2 group than in Type 1 patients (Table 5.2). Duration of diabetes until onset of retinopathy and hypertension was also shorter in the Type 2 sub-group in comparison to the Type 1 sub-group and these differences were statistically significant. The statistical power of the sub-study, calculated after collection of data, was low (<18%), therefore, this sub-study could be regarded as a pilot study. In order to achieve 80% power a minimum of 780 patients would be needed.

Complication	Time to Onset Mean (±SD) [years] Type 1	Time to Onset Mean(±SD) [years] Type 2	*P*
Retinopathy	17(6)	11(6)	<0.001
Proteinuria	20(8)	14(6)	<0.001
Hypertension	21(8)	13(4)	<0.001

SD: Standard Deviation Level of significance: $P \leq 0.05$ DM: Diabetes mellitus

Table 5.2: Duration of diabetes to onset of complications

However, the medians for duration of diabetes to onset of all three complications were shorter than the means in the Type 1 sub-group while the range was also larger than the Type 2 sub-group (Figure 5.1).

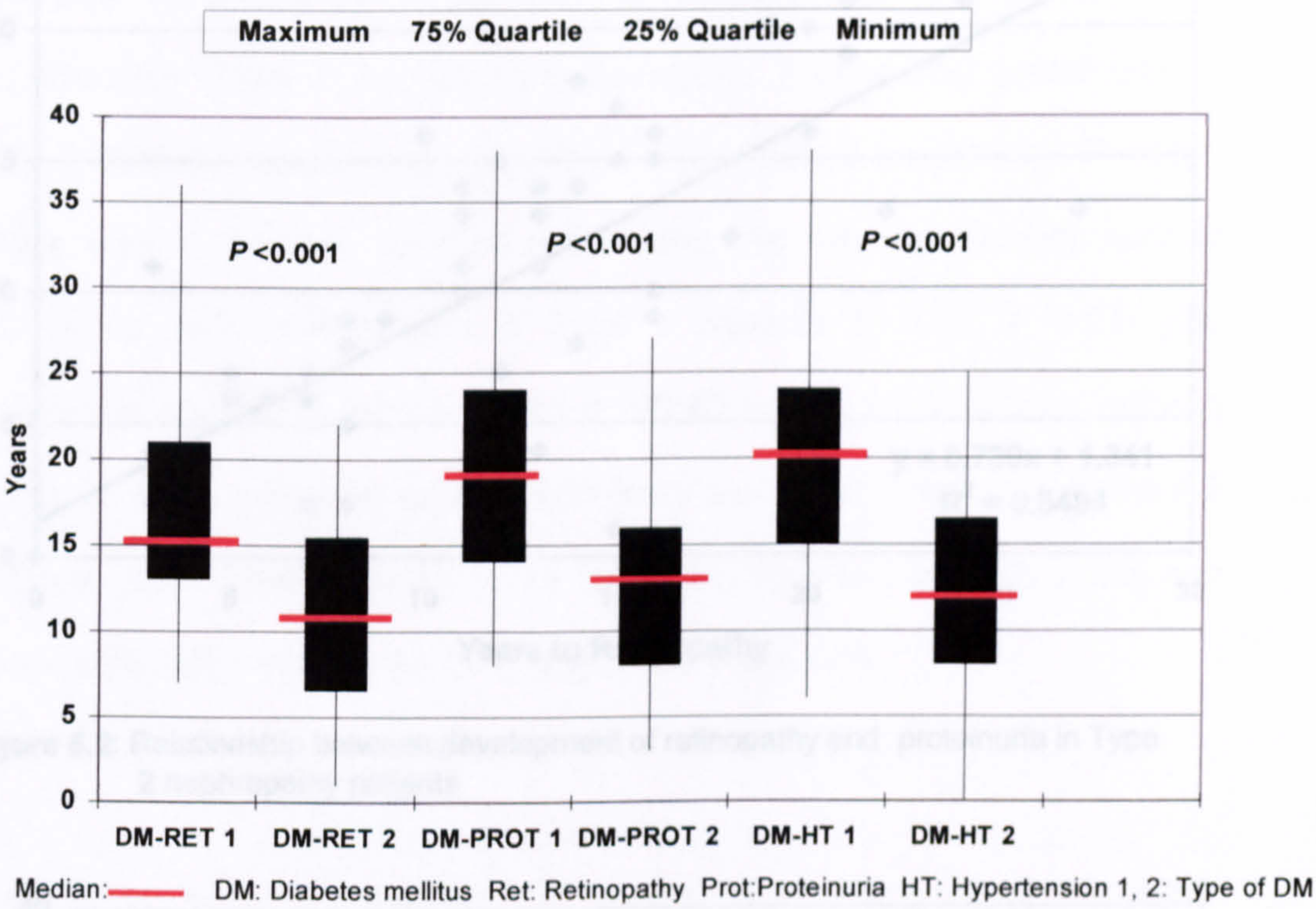


Figure 5.1: Comparison of time to onset of complications: Type 1 versus Type 2 nephropathy patients.

The difference of six years in the mean length of time between development of retinopathy (Type 1: 17 (±8) years compared to Type 2: 11 (6±) years) was statistically significant (P<0.001), as was the difference of six years in mean length of time to development of proteinuria (Type 1: 20 (±8) years versus Type 2: 14 (±6) years) (P<0.001). When these differences were compared between the two sub-groups, Type 1 patients had retinopathy for 1 year longer than Type 2 patients before developing proteinuria (P<0.001). There was a positive correlation between these two parameters in Type 2 patients (r=0.74, P<0.001) but less so in Type 1 patients (r=0.65, P<0.001)

(one outlying point was removed which increased the correlation coefficient from r=0.52 to r=0.65) (Figure 5.2 and 5.3).

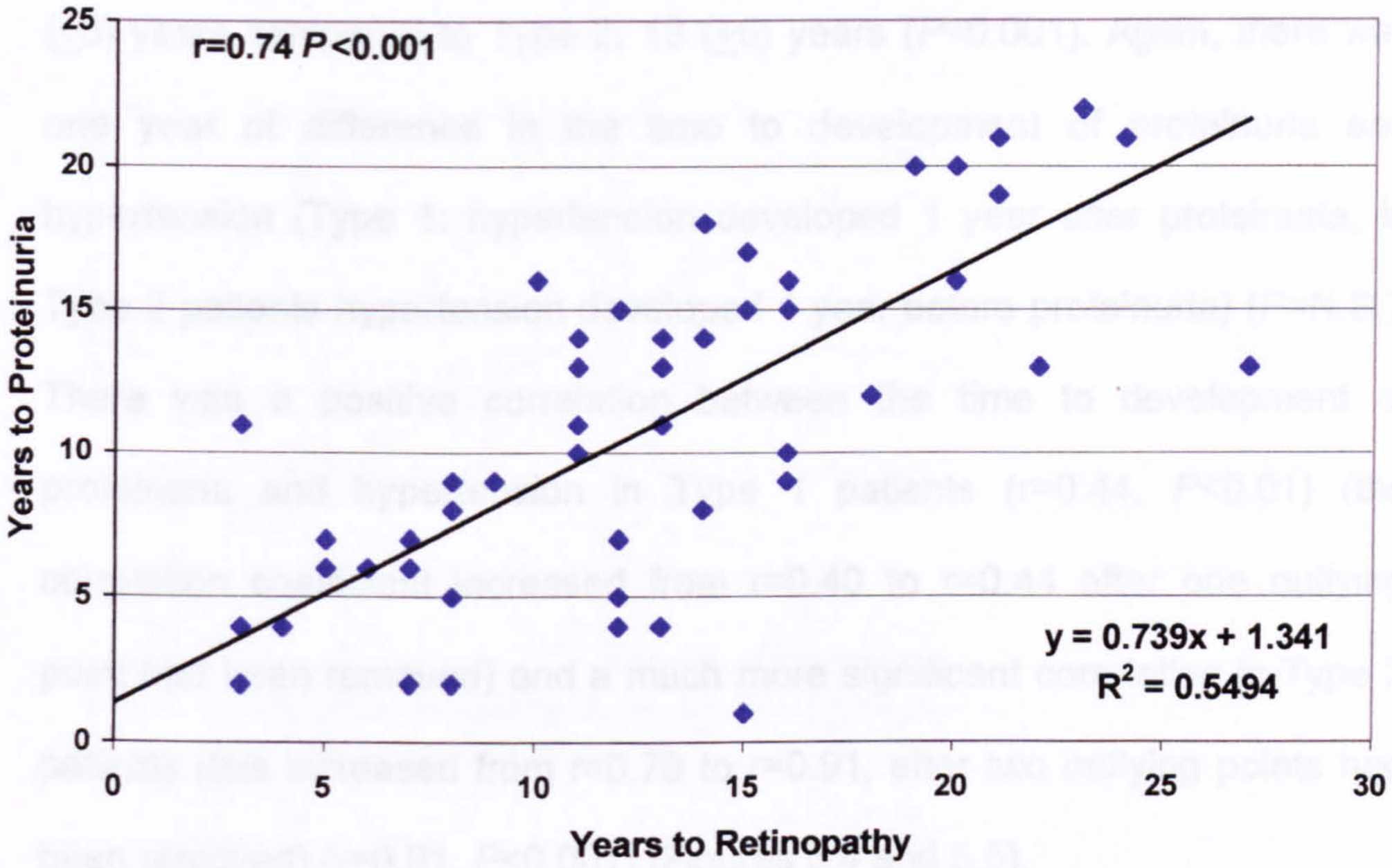


Figure 5.2: Relationship between development of retinopathy and proteinuria in Type 2 nephropathy patients

Figure 5.3: Relationship between development of retinopathy and proteinuria in Type1 nephropathy patients

There was a significant difference in the mean length of time from diagnosis of diabetes to development of hypertension between the groups: Type 1: 21 (±8) years compared to Type 2: 13 (±6) years (P<0.001). Again, there was one year of difference in the time to development of proteinuria and hypertension (Type 1: hypertension developed 1 year after proteinuria; in Type 2 patients hypertension developed 1 year before proteinuria) (P=N.S.). There was a positive correlation between the time to development of proteinuria and hypertension in Type 1 patients (r=0.44, P<0.01) (the correlation coefficient increased from r=0.40 to r=0.44 after one outlying point had been removed) and a much more significant correlation in Type 2 patients (this increased from r=0.79 to r=0.91, after two outlying points had been removed) (r=0.91, P<0.001) (Figures 5.4 and 5.5).

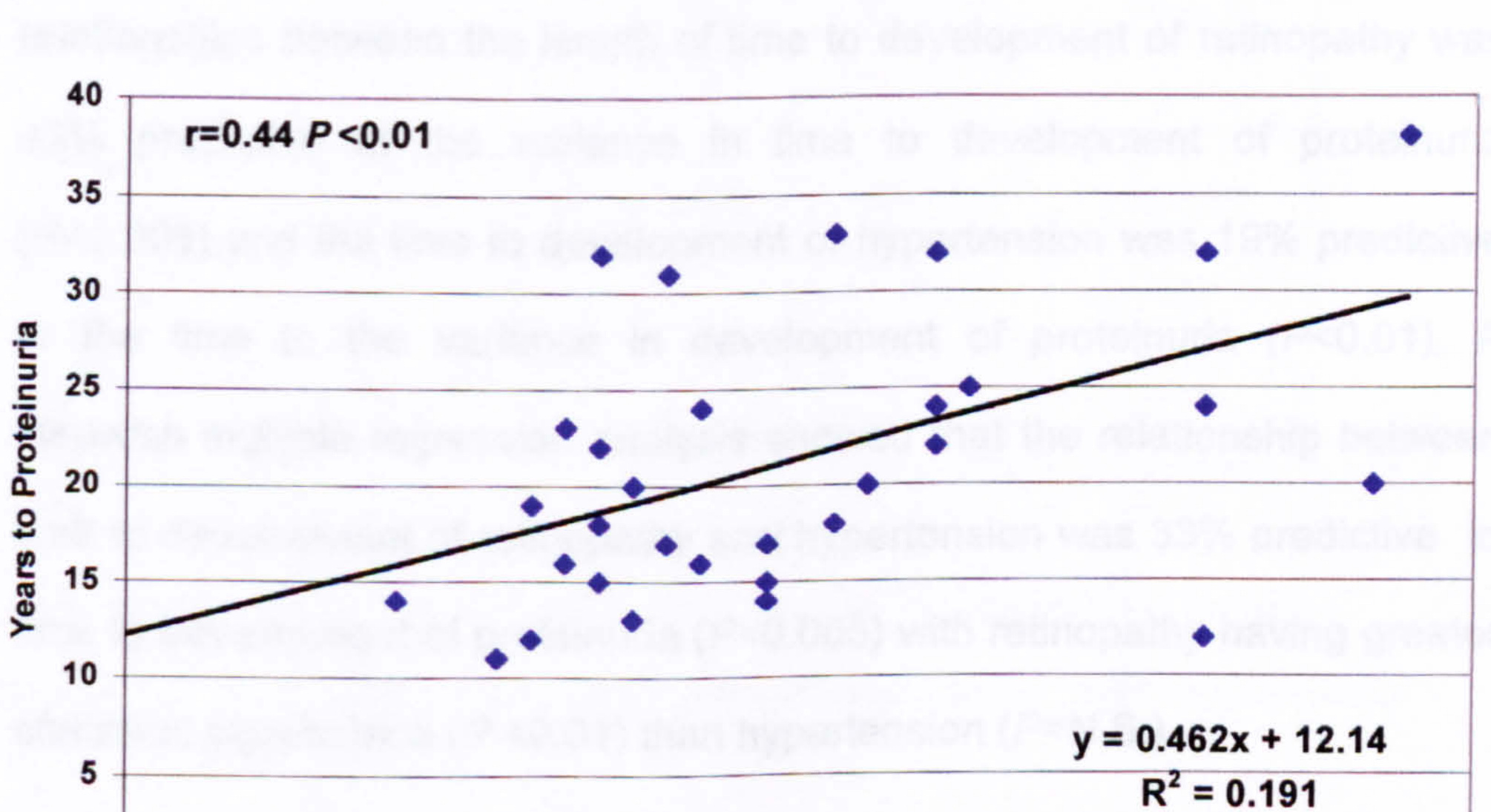


Figure 5.4: Relationship between the development of hypertension and proteinuria in Type 1 nephropathy patients

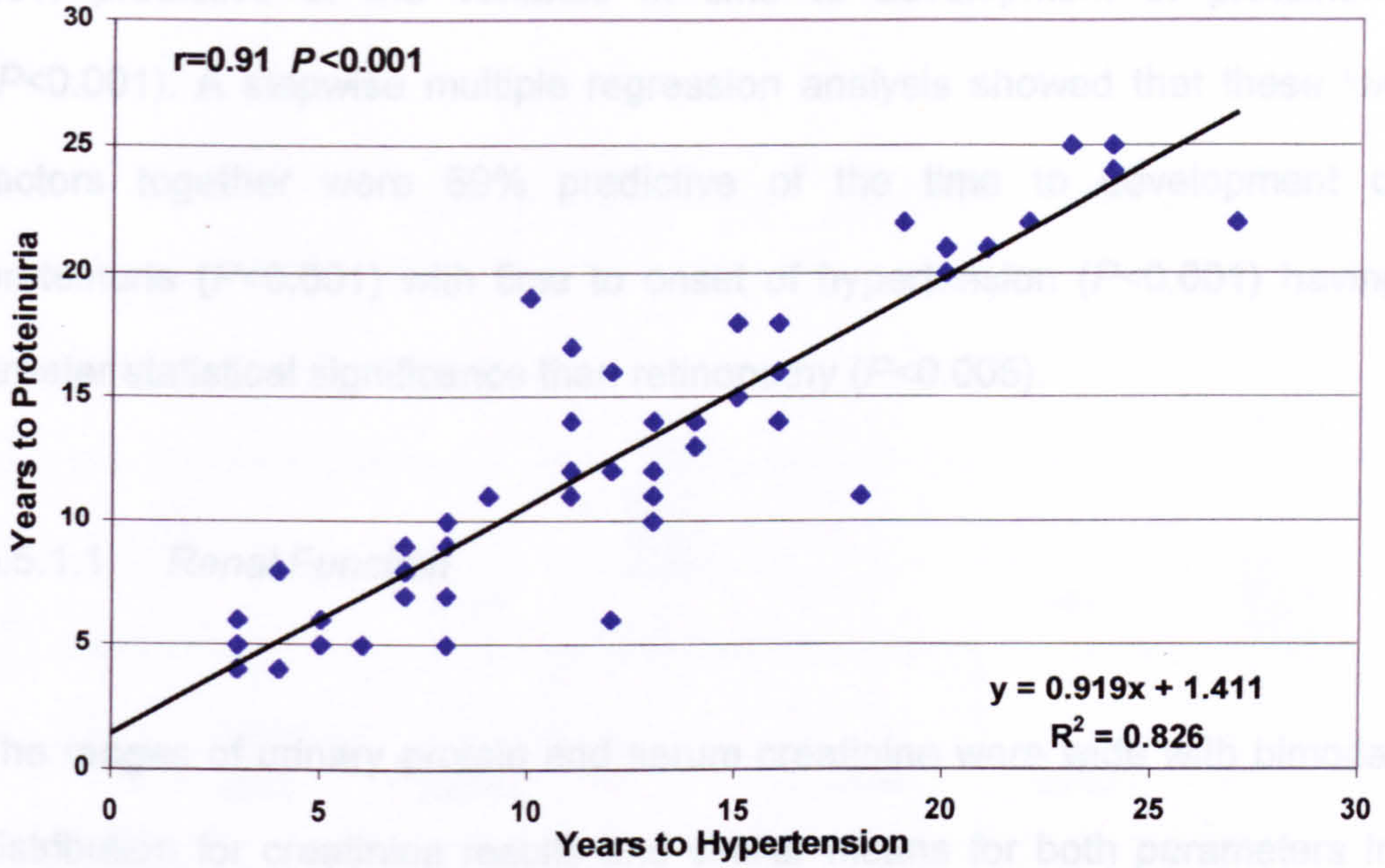


Figure 5.5: Relationship between development of hypertension and proteinuria in Type 2 nephropathy patients

In Type 1 patients linear regression analysis demonstrated that the individual relationships between the length of time to development of retinopathy was 43% predictive of the variance in time to development of proteinuria ($P<0.001$) and the time to development of hypertension was 19% predictive of the time to the variance in development of proteinuria ($P<0.01$). A stepwise multiple regression analysis showed that the relationship between time to development of retinopathy and hypertension was 33% predictive to time to development of proteinuria ($P<0.005$) with retinopathy having greater statistical significance ($P<0.01$) than hypertension (P=N.S.).

In Type 2 patients the individual relationships between time to development of retinopathy was 55% predictive of the variance in time to development of proteinuria ($P<0.001$) while the time to development of hypertension was

83% predictive of the variance in time to development of proteinuria (*P*<0.001). A stepwise multiple regression analysis showed that these two factors together were 69% predictive of the time to development of proteinuria (*P*<0.001) with time to onset of hypertension (*P*<0.001) having greater statistical significance than retinopathy (*P*<0.005).

5.5.1.1 *Renal Function*

The ranges of urinary protein and serum creatinine were wide with bimodal distribution for creatinine results and similar means for both parameters in both groups (*P*=N.S.). However, the medians were much lower than the means (Table 5.3).

	Normal Range	Type 1	Type 2	*P*
SERUM CREATININE (μmol l^{-1})				
Range	60-120	69-774	65-680	
Mean (±SD)		188 (187)	183 (137)	N.S.
Median		105	131	
PROTEINURIA (g 24hours^{-1})				
Range	0.00-0.10	0.10-16.00	0.07-21.00	
Mean (±SD)		3.06 (3.28)	2.72 (3.46)	N.S.
Median		1.96	1.79	

N.S.: Not significant Level of significance: $P \leq 0.05$ SD: standard deviation

Table 5.3: Renal function: Type 1 versus Type 2 patients with nephropathy

When the "outliers" were removed from the creatinine results (revised results) the means were greatly reduced in both groups (*P*=N.S.) while the medians were lower than previously (Figure 5.6). The results for the "outliers", clearly showed that these Type 1 patients were in end stage renal

failure (ESRF) and that the Type 2 group were progressing towards ESRF (P<0.05) (Figure 5.6).

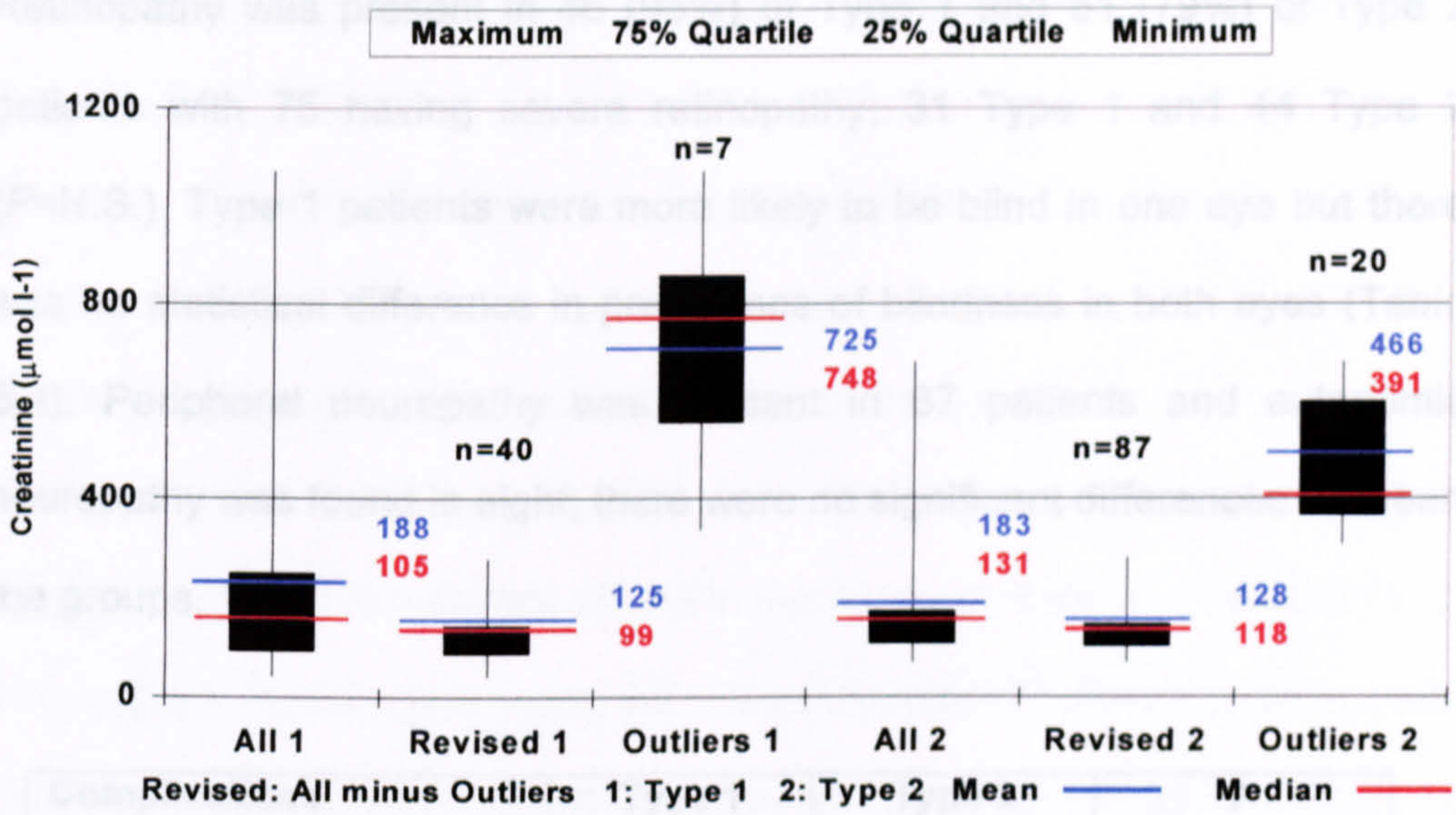


Figure 5.6: Comparison of creatinine levels in nephropathy patients

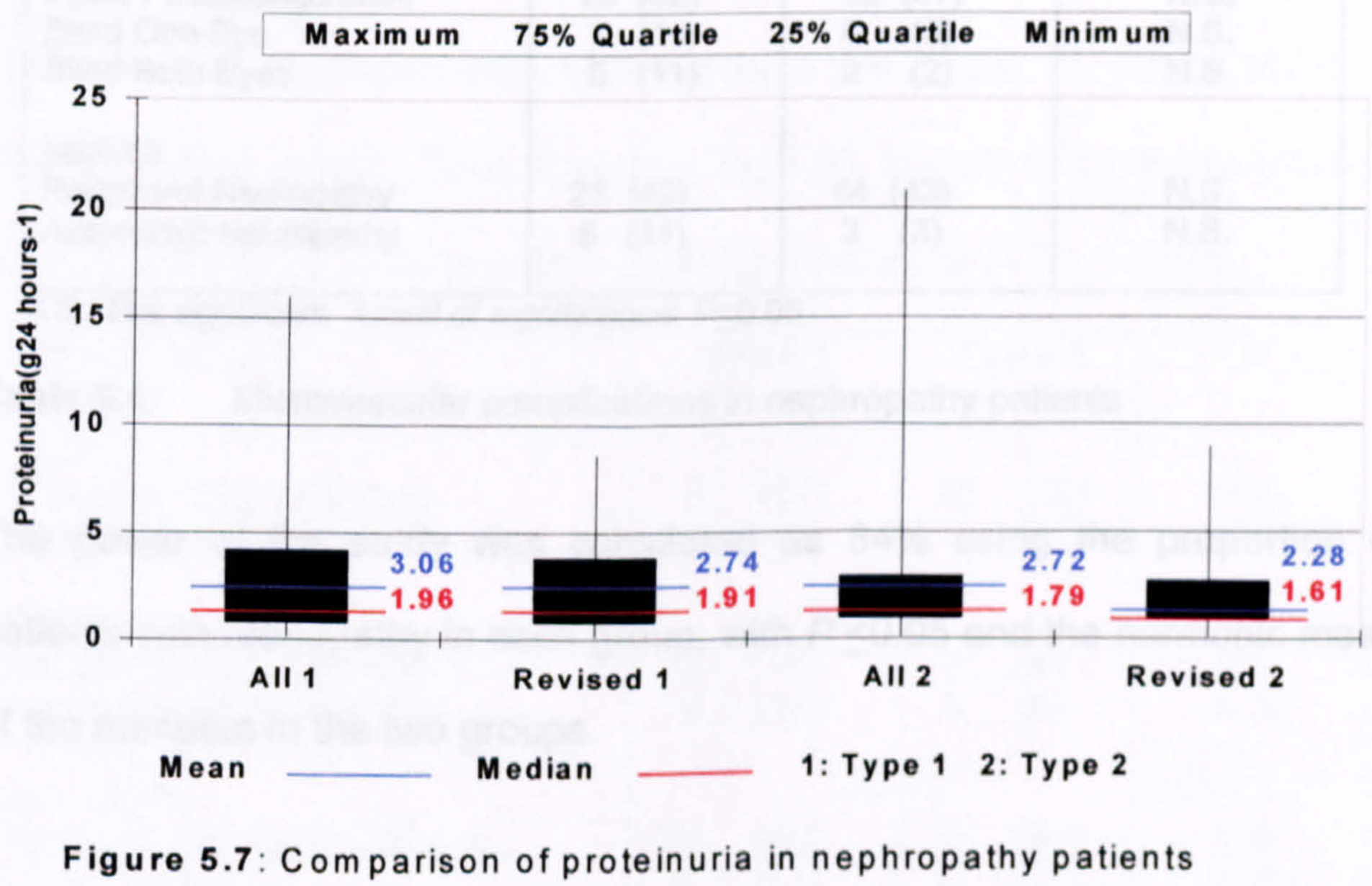


Figure 5.7: Comparison of proteinuria in nephropathy patients

The revised proteinuria results demonstrated a smaller reduction in the means and medians for both groups (P = N.S.)(Figure 5.7).

5.5.1.2 Microvascular Complications

Retinopathy was present in 46 (96%) of Type 1 and 81 (79%) of Type 2 patients with 75 having severe retinopathy: 31 Type 1 and 44 Type 2 (*P*=N.S.). Type 1 patients were more likely to be blind in one eye but there was no statistical difference in prevalence of blindness in both eyes (Table 5.4). Peripheral neuropathy was present in 67 patients and autonomic neuropathy was found in eight; there were no significant differences between the groups.

Complications	**Type 1 n (%)**	**Type 2 n (%)**	***P***
EYES			
Retinopathy	46 (96)	81 (79)	N.S.
Laser Photocoagulation	29 (62)	38 (37)	N.S.
Blind One Eye	7 (14)	6 (6)	N.S.
Blind Both Eyes	5 (11)	2 (2)	N.S.
NERVES			
Peripheral Neuropathy	23 (49)	44 (43)	N.S.
Autonomic Neuropathy	5 (11)	3 (3)	N.S.

N.S.: Not significant Level of significance: $P \leq 0.05$

Table 5.4: Microvascular complications in nephropathy patients

The power of the study was calculated as 84% using the proportion of patients with retinopathy in each group, with $P \leq 0.05$ and the harmonic mean of the numbers in the two groups.

5.5.1.3 Macrovascular Complications

There were no statistically significant differences in macrovascular complications between the groups, 28 patients had evidence of IHD and 15 had had a myocardial infarction. Six patients had experienced transient ischaemic attacks and 14 had had cerebrovascular accidents. Fifty-two patients had peripheral vascular disease, in 19 cases this was severe, 22 had leg or foot ulcers and five had one limb amputated (Table 5.5). Total serum cholesterol levels were raised in both groups; Type 1: mean 6.5 (SD± 2.0) mmol l^{-1} and Type 2: mean 6.7 (SD± 2.0) mmol l^{-1} (*P*=N.S.). Body mass index indicated that both groups were overweight: Type 1: 26.1 (SD± 5) kg m^{-2}, Type 2: 28.3 (SD±4) kg m^{-2} (*P*=N.S.).

There was no significant difference between the groups for family history of diabetes (36% v 36%: Type 1 versus Type 2), history of cigarette smoking (55% v 50%) or in defaulting from diabetic clinic visits (47% v 39%).

Complications	Type 1 n (%)	Type 2 n (%)	*P*
HEART			
Ischaemic Heart Disease	8 (17)	20 (20)	N.S.
Myocardial Infarction	3 (6)	12 (12)	N.S.
BRAIN			
Transient Ischaemic Attack	2 (4)	4 (4)	N.S.
Cerebrovascular Accident	6 (13)	8 (8)	N.S.
VASCULAR			
Peripheral Vascular Disease	19 (40)	33 (32)	N.S.
Ulcer Leg/Feet	5 (11)	17 (17)	N.S.
Amputation (1 leg)	3 (6)	2 (2)	N.S.

N.S.: Not significant Level of significance: $P \leq 0.05$

Table 5.5: Macrovascular complications in nephropathy patients

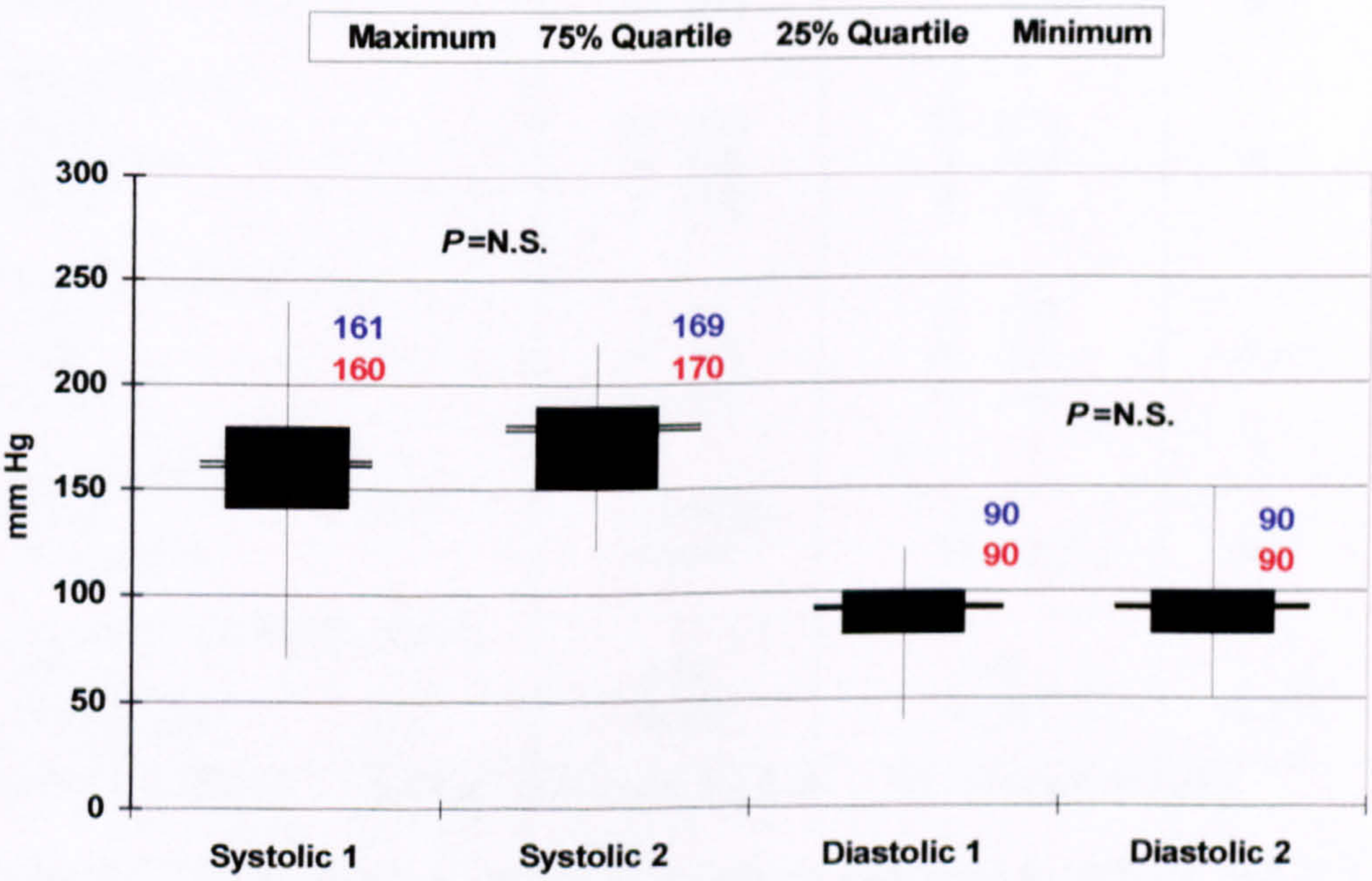
Hypertension was found in 140 patients, yet only 64 were on anti-hypertensive medication at referral (Table 5.6).

	Type 1 n (%)	Type 2 n (%)	*P*
HYPERTENSION	44 (93)	96 (94)	N.S.
ANTI-HYPERTENSIVE THERAPY	20 (45)	44 (46)	N.S.
SYSTOLIC PRESSURE (mm Hg)			
Range	70-240	120-220	
Mean (±SD)	161(±29)	168 (±25)	N.S.
DIASTOLIC PRESSURE (mm Hg)			
Range	40-120	50-150	
Mean (±SD)	90 (±15)	90 (±15)	N.S.

N.S.: Not significant Level of significance: $P \leq 0.05$ SD: Standard deviation

Table 5.6: Blood pressure and treatment: Type 1 versus Type 2 nephropathy patients

Both groups had systolic hypertension with a wide range in Type 1 patients (Figure 5.8).



Mean, Median, 1: Type 1, 2: Type 2, N.S.: Not significant Level of significance: $P \leq 0.05$

Figure 5.8: Blood pressure: Type 1 versus Type 2 nephropathy patients

The mean diastolic pressure was at the upper limit of normal in both groups, with a wider range in the Type 2 group. Less than half of each group with hypertension were receiving anti-hypertensive therapy at referral. There was no significant difference in mean systolic or diastolic pressures between the groups.

5.5.1.4 Progression of Renal Disease

Over an eight year period, thirty-five patients reached ESRF and received renal replacement therapy (RRT); 16 (34%) Type 1 (11 CAPD and 5 HD) and 19 (19%) Type 2 (13 CAPD and 6 HD), nine patients received renal transplants (*P*=N.S.).

5.5.2. *Comparison of Type 2 Patients with Nephropathy and Type 2 Patients with Non-Diabetic Renal Disease.*

Sixty-two Type 2 patients with nephropathy were compared to sixty-nine Type 2 patients with renal disease due to causes other than diabetic nephropathy (other causes were described in Chapter 4.5). Diagnosis was confirmed by biopsy in five nephropathy patients and in 11 patients with non-diabetic renal disease (*P*=N.S.). Patients in both groups were predominantly male and White. However, there was a higher proportion of Indo-Asian and Black patients in the nephropathy group (Table 5.7).

	Nephropathy n (%)	**Non-Diabetic Renal Disease n (%)**	***P***
TOTAL NUMBER	62 (100)	69 (100)	
GENDER			
Male	41 (66)	38 (55)	N.S.
Female	21 (34)	31 (45)	N.S.
ETHNIC GROUP			
White	36 (58)	55 (80)	
Indo-Asian	18 (29)	8 (11)	<0.05
Black	8 (13)	6 (9)	
DIABETIC TREATMENT			
Diet	1 (1)	12 (17)	
Oral	11 (18)	40 (58)	<0.001
Insulin	50 (81)	17 (25)	
AGE (Years)			
Range	41-81	31-77	
Mean (±SD)	61(±8)	64(±9)	N.S.
DURATION OF DIABETES (Years)			
Range	4-28	1-40	
Mean (±SD)	15(±6)	9(±8)	<0.001

N.S.: Not significant Level of significance: $P \leq 0.05$ SD: Standard deviation

Table 5.7: Demographics of nephropathy patients compared to patients with non diabetic renal disease

More of the nephropathy group were treated with insulin (81% versus 25%) ($P<0.001$) and diabetic control was worse in the nephropathy group; the mean (±SD) HbA1 for nephropathy patients was 11.6 (±2.6)% versus 10.6 (±2.4)% in the other group (P=N.S.). Both groups were overweight with the same mean body mass index: 29 kg m^{-2}.

The power of the study was calculated as 99% using the proportion of patients in each group receiving insulin treatment (Type 1: 0.81 and Type 2: 0.25), $P \leq 0.05$ and the harmonic mean of the numbers in each group (65).

There was a significant difference in duration of diabetes at referral between the groups (Table 5.7). When duration of diabetes to the development of retinopathy was compared there was no statistical difference between the groups (Figure 5.9).

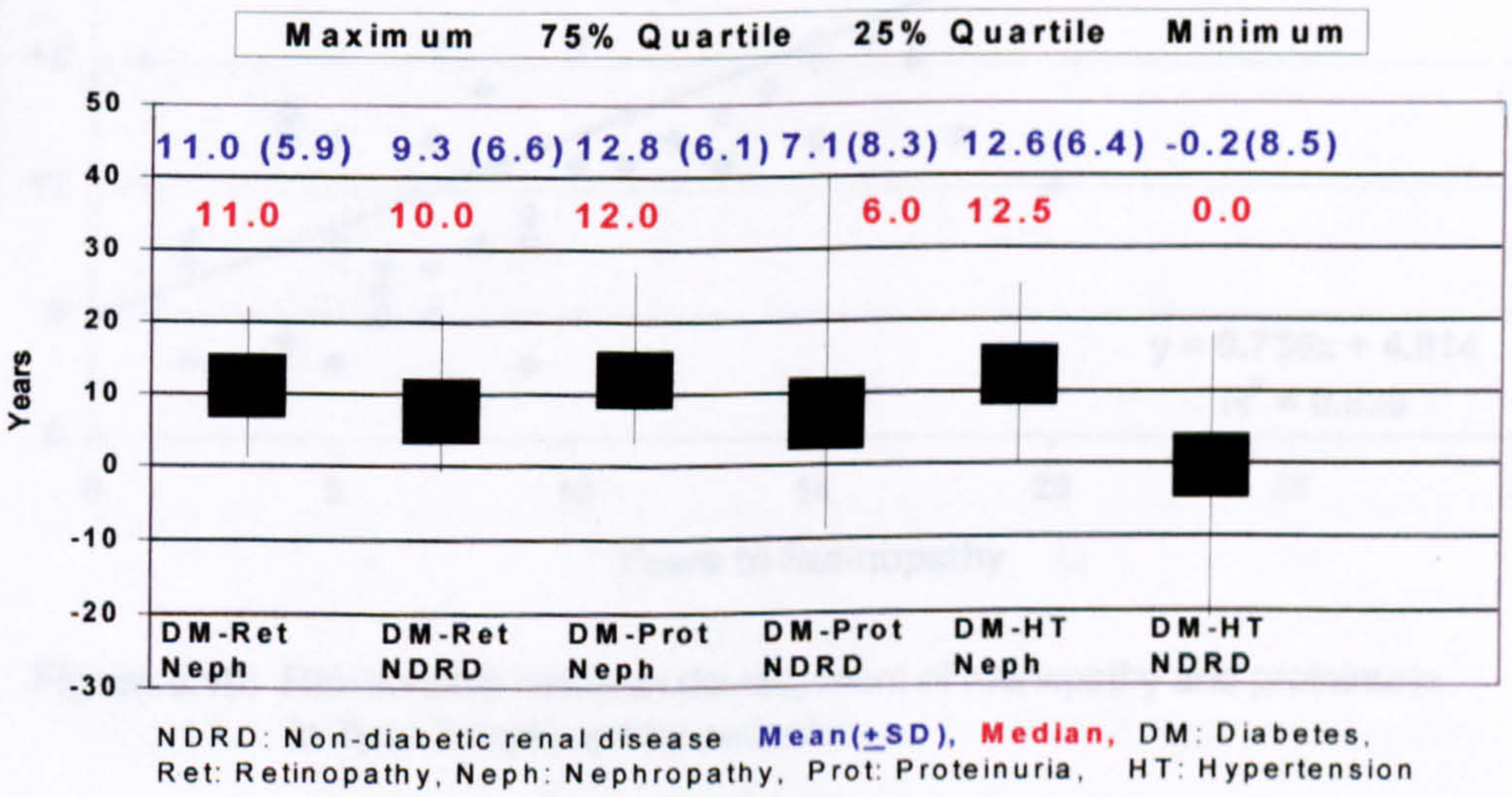


Figure 5.9: Time to development of complications: Type 2 nephropathy group versus Type 2 patients with non-diabetic renal disease

Duration of diabetes to onset of proteinuria was longer in the nephropathy group (mean: 12.8 (±6.1) years) than in the non-diabetic renal disease group (mean: 7.1(±8.3) years) and this was significant (P<0.001). There was also a significant difference in duration of diabetes to onset of hypertension (nephropathy group; mean: 12.6 (±6.4) years versus non-diabetic renal disease; mean: -0.2 (±8.0) years) (P<0.001).

There was a strong positive correlation between time to development of retinopathy and proteinuria (Figures 5.10 and 5.11) and between time to onset of hypertension and proteinuria in both groups (Figures 5.12 and 5.13) (P<0.001 for each correlation).

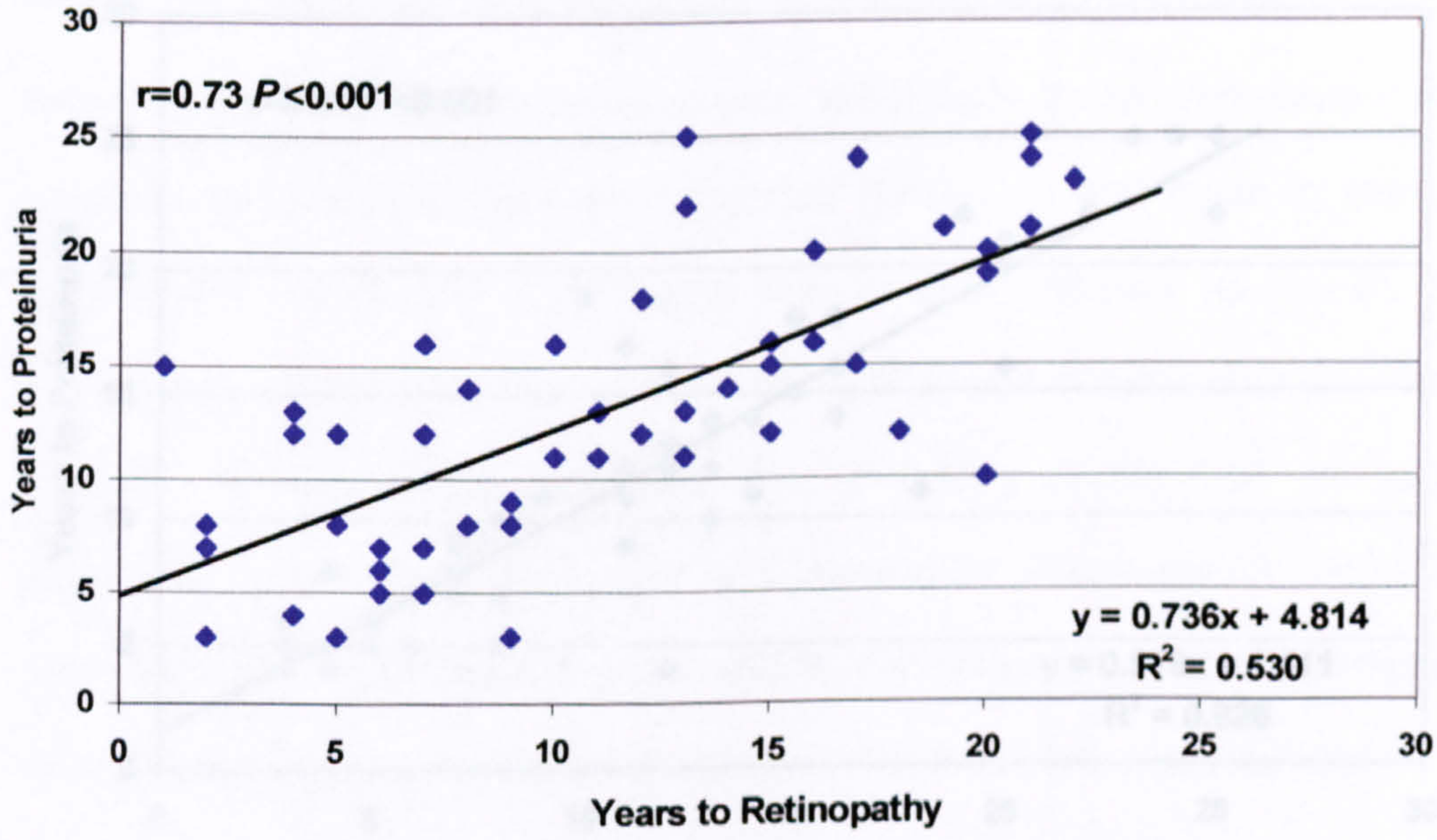


Figure 5.10: Relationship between development of retinopathy and proteinuria in Type 2 nephropathy patients

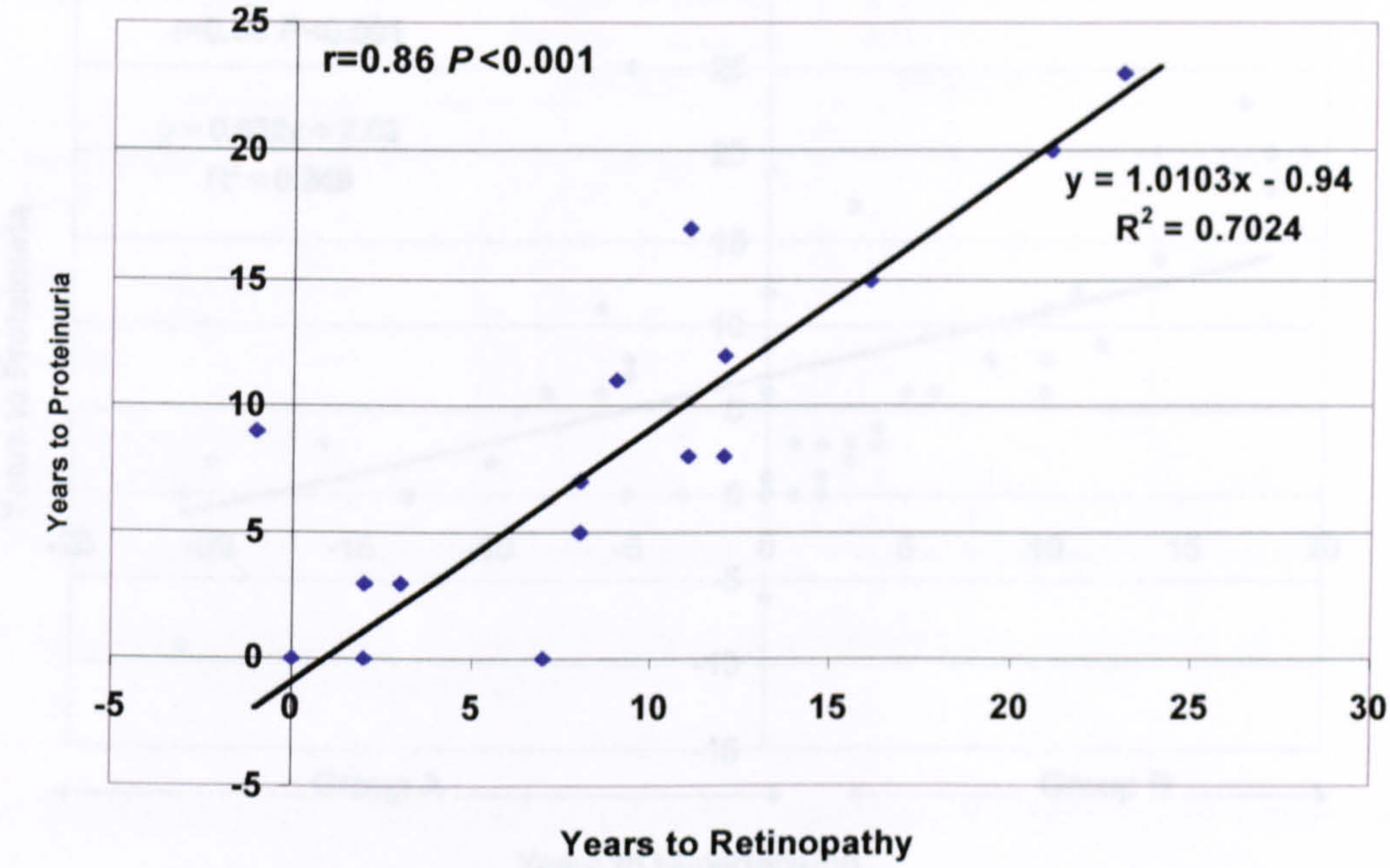


Figure 5.11: Relationship between development of retinopathy and proteinuria in Type 2 patients with non-diabetic renal disease

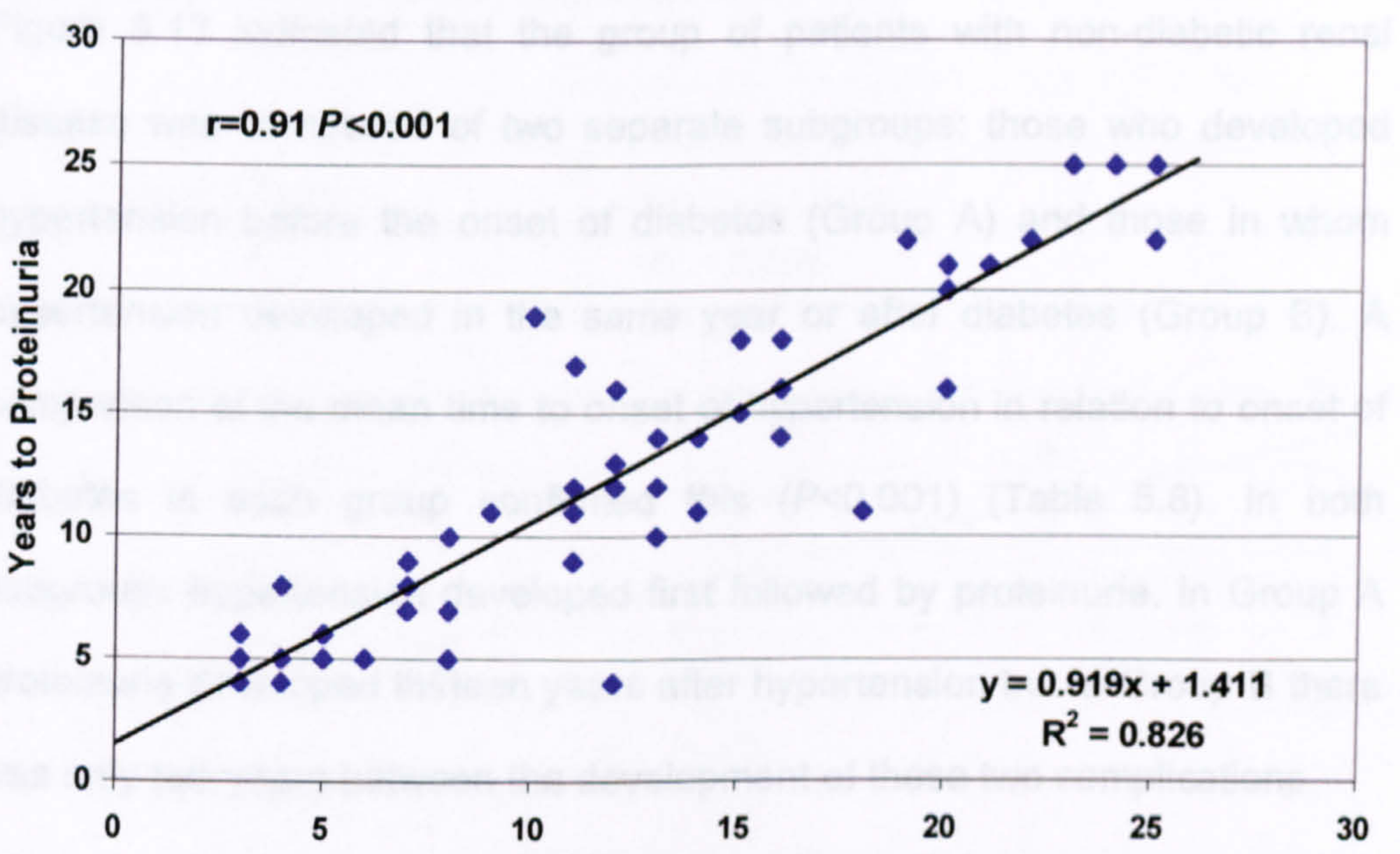


Figure 5.12: Relationship between development of hypertension and proteinuria in Type 2 nephropathy patients

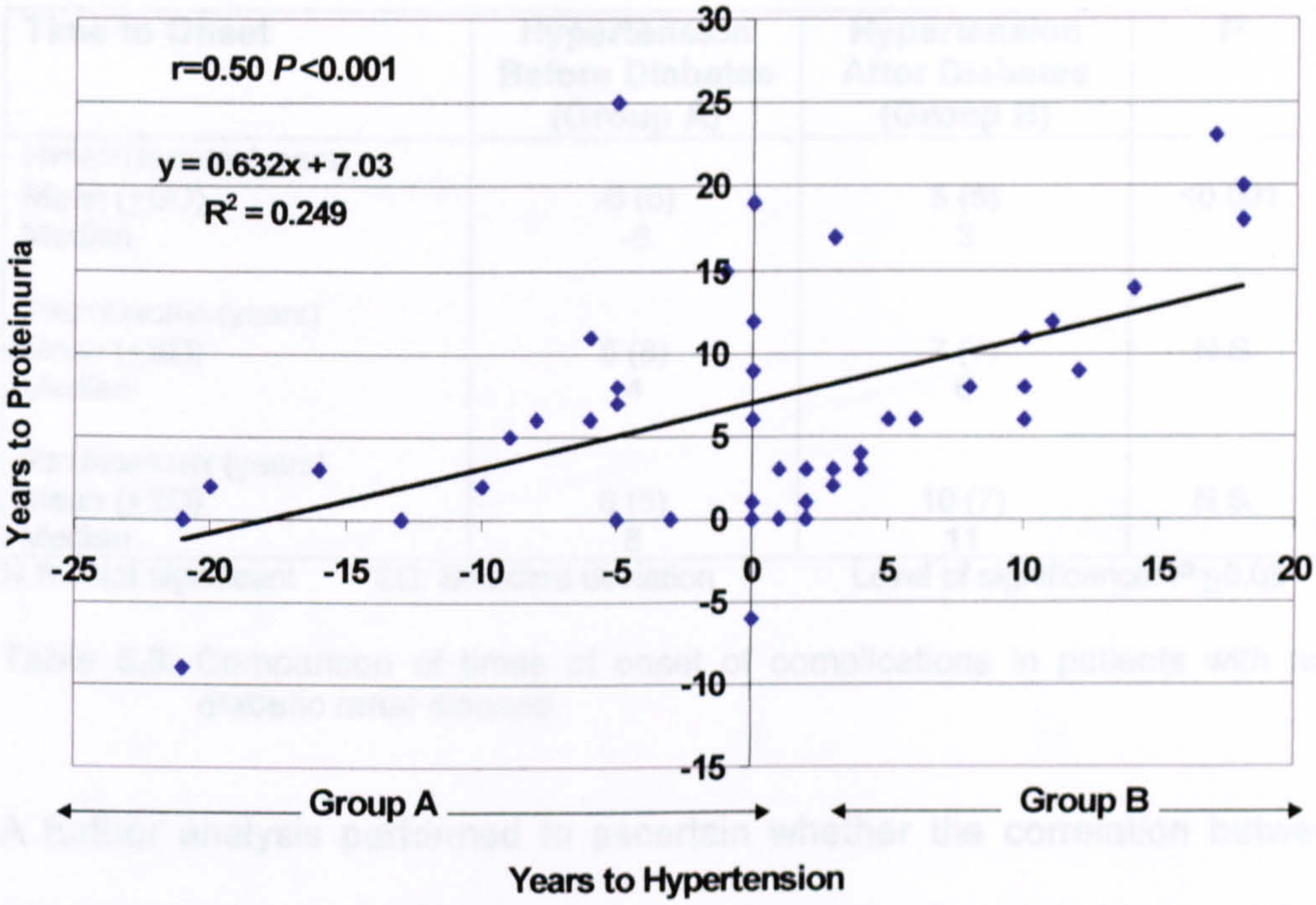


Figure 5.13: Relationship between development of hypertension and proteinuria in Type 2 patients with non-diabetic renal disease

Figure 5.13 indicated that the group of patients with non-diabetic renal disease was composed of two separate subgroups: those who developed hypertension before the onset of diabetes (Group A) and those in whom hypertension developed in the same year or after diabetes (Group B). A comparison of the mean time to onset of hypertension in relation to onset of diabetes in each group confirmed this (P<0.001) (Table 5.8). In both subgroups hypertension developed first followed by proteinuria. In Group A proteinuria developed thirteen years after hypertension but in Group B there was only two years between the development of these two complications.

Time to Onset	Hypertension Before Diabetes (Group A)	Hypertension After Diabetes (Group B)	*P*
HYPERTENSION (years)			
Mean (±SD)	-8 (6)	5 (5)	<0.001
Median	-6	3	
PROTEINURIA (years)			
Mean (±SD)	5 (8)	7 (7)	N.S.
Median	4	6	
RETINOPATHY (years)			
Mean (±SD)	6 (5)	10 (7)	N.S.
Median	8	11	

N.S.: Not significant SD: Standard deviation Level of significance: $P \leq 0.05$

Table 5.8: Comparison of times of onset of complications in patients with non-diabetic renal disease

A further analysis performed to ascertain whether the correlation between time to development of hypertension and proteinuria existed separately in these two subgroups showed a positive correlation between these two parameters in both groups (Figures 5.14 and 5.15).

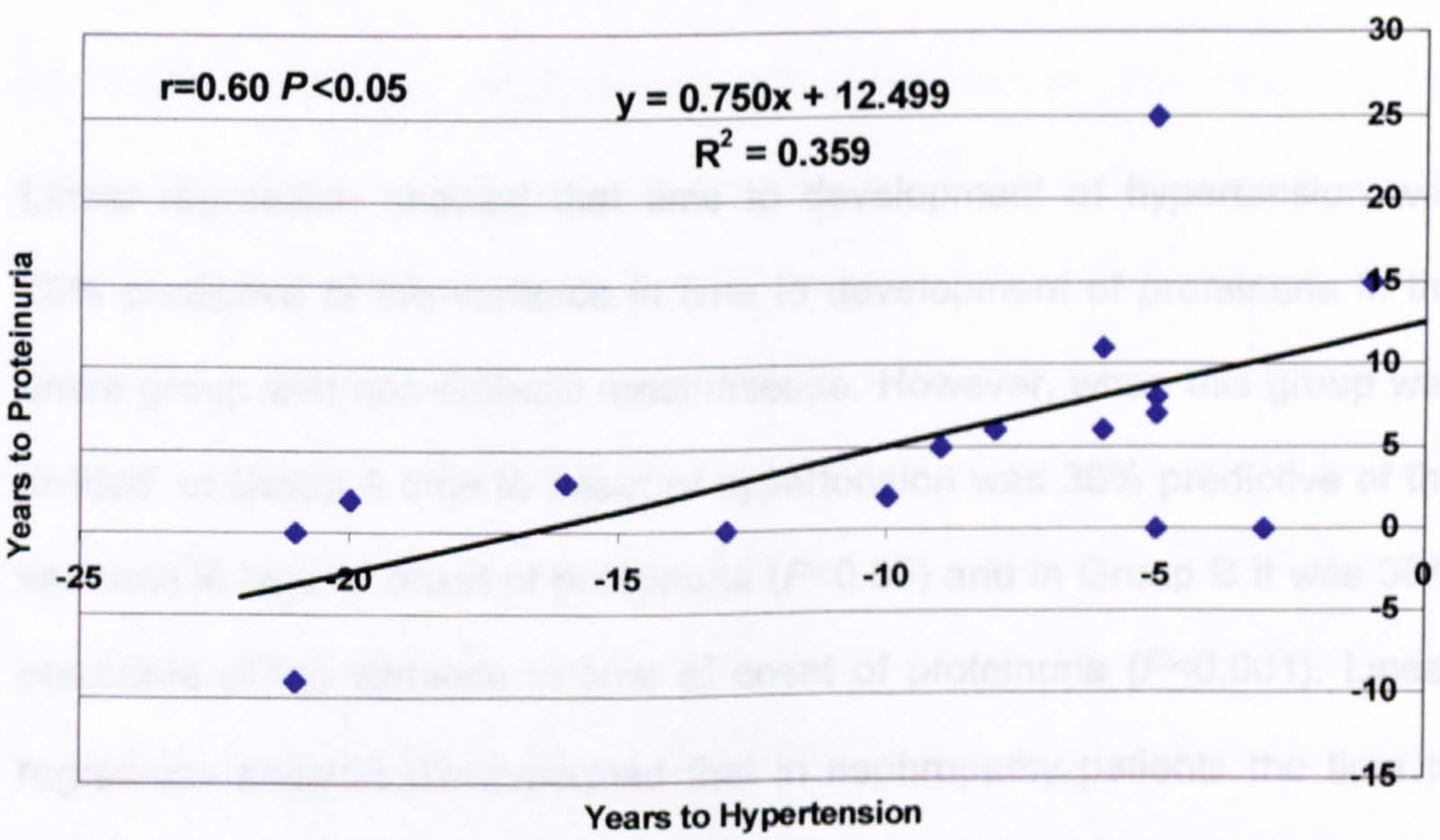


Figure 5.14: Relationship between development of hypertension and proteinuria in patients with non-diabetic renal disease where hypertension developed before onset of diabetes (n=16) (Group A)

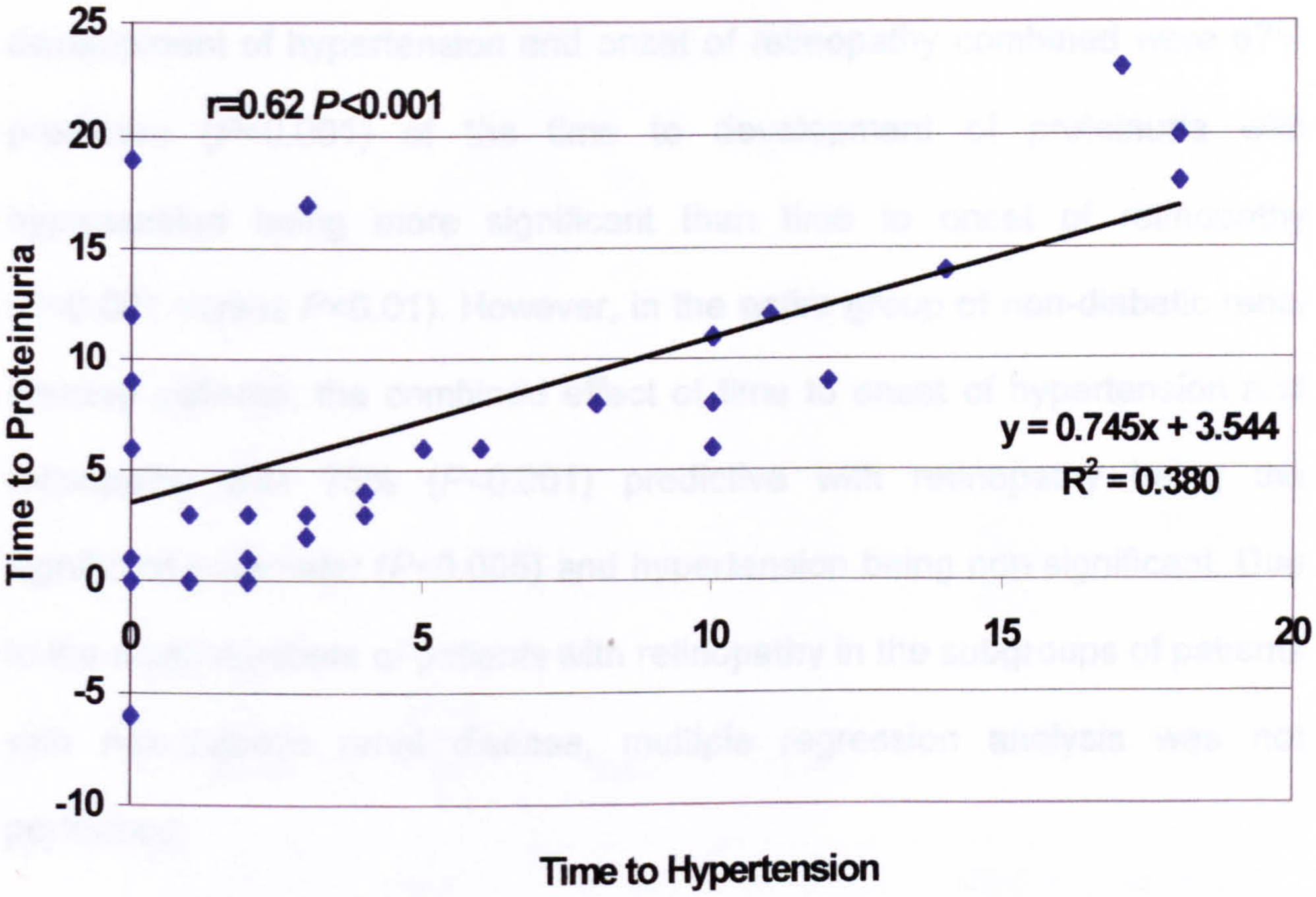


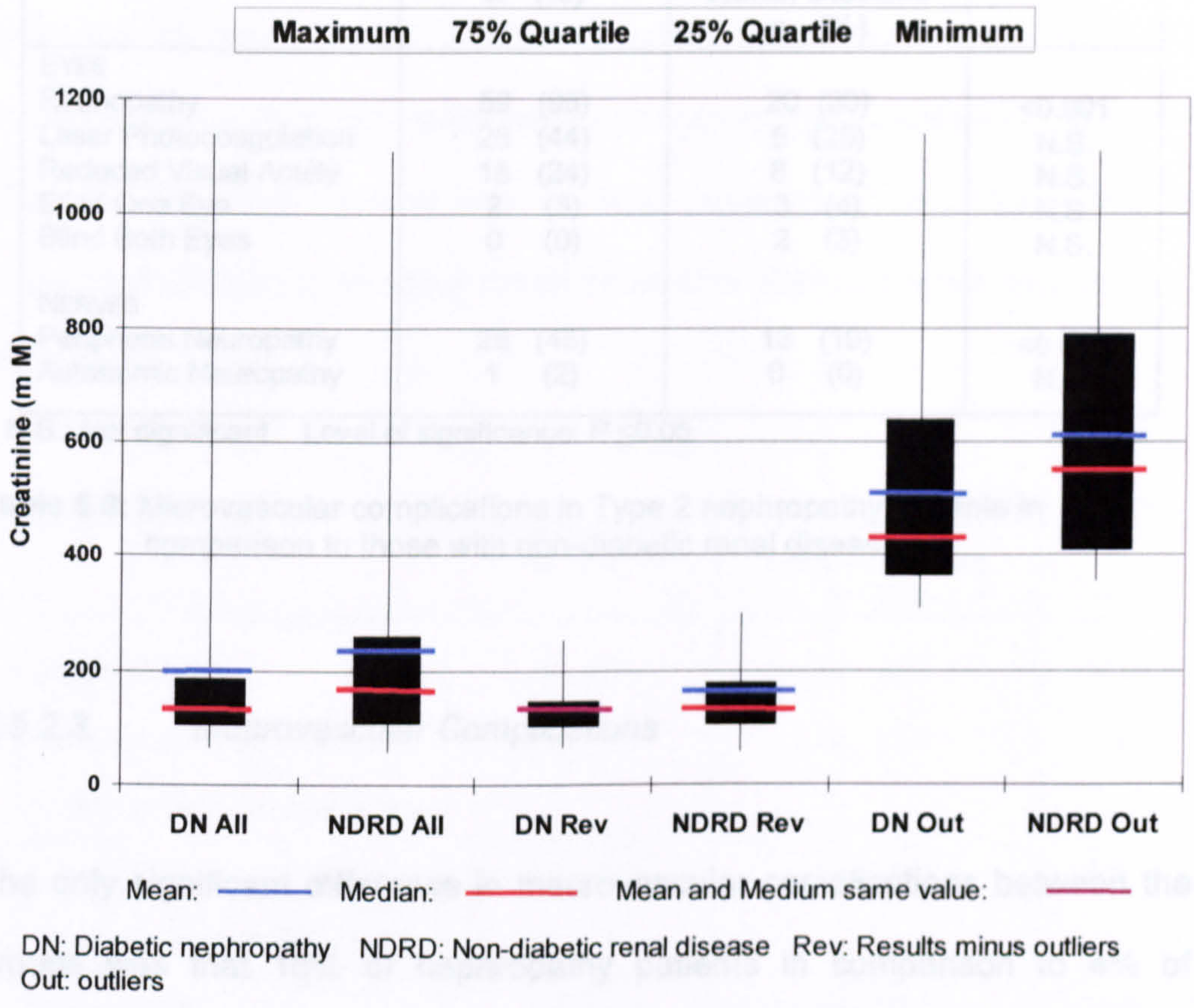
Figure 5.15: Relationship between development of hypertension and proteinuria in patients with non-diabetic renal disease where hypertension has developed in the same year or after diabetes (n=34) (Group B)

Linear regression showed that time to development of hypertension was 25% predictive of the variance in time to development of proteinuria in the entire group with non-diabetic renal disease. However, when this group was divided, in Group A time to onset of hypertension was 36% predictive of the variance in time to onset of proteinuria ($P<0.05$) and in Group B it was 38% predictive of the variance in time of onset of proteinuria ($P<0.001$). Linear regression analysis demonstrated that in nephropathy patients the time to development of retinopathy was 53% predictive ($P<0.001$) and in patients with non-diabetic renal disease was 70% ($P<0.001$) predictive of the variance in time to development of proteinuria ($P<0.001$).

Multiple regression analysis showed that in nephropathy patients time to development of hypertension and onset of retinopathy combined were 67% predictive ($P<0.001$) of the time to development of proteinuria with hypertension being more significant than time to onset of retinopathy ($P<0.001$ versus $P<0.01$). However, in the entire group of non-diabetic renal disease patients, the combined effect of time to onset of hypertension and retinopathy was 75% ($P<0.001$) predictive with retinopathy being the significant parameter ($P<0.005$) and hypertension being non significant. Due to the small numbers of patients with retinopathy in the subgroups of patients with non-diabetic renal disease, multiple regression analysis was not performed.

5.5.2.1 Renal Function

The ranges of serum creatinine were wide in both groups. The mean and the median for the non-diabetic renal disease group (all patients) was higher than in the nephropathy group but this was not statistically significant (Figures 5.16). After removal of outliers in each group, there was still no significant difference between the groups both in the revised and outliers results (Figures 5.16). The outliers represented patients in ESRF and the revised group represented those in chronic renal failure. There were wide ranges of proteinuria in both groups although the means were the same (nephropathy group range: 0.09-13.30 g $24h^{-1}$, mean: 2.83 g $24h^{-1}$ and the non-diabetic nephropathy group range: 0.07-21.00 g $24h^{-1}$: mean of 2.90 g $24h^{-1}$) (P=N.S.).



DN: Diabetic nephropathy NDRD: Non-diabetic renal disease Rev: Results minus outliers
Out: outliers

Figure 5.16: Comparison of serum creatinine concentration in Type 2 patients with nephropathy and those with non-diabetic renal disease

5.5.2.2 Microvascular Complications

There were two differences in point prevalence of microvascular complications between the groups. Ninety-five percent of nephropathy patients in comparison to only 30% of patients with non-diabetic renal disease had retinopathy ($P<0.001$), while 45% of the nephropathy group had peripheral neuropathy in comparison to 19% of non-nephropathy patients ($P<0.005$) (Table 5.9).

	Nephropathy n (%)	Non-Diabetic Renal Disease n (%)	*P*
EYES			
Retinopathy	59 (95)	20 (30)	<0.001
Laser Photocoagulation	26 (44)	5 (25)	N.S.
Reduced Visual Acuity	15 (24)	8 (12)	N.S.
Blind One Eye	2 (3)	3 (4)	N.S.
Blind Both Eyes	0 (0)	2 (3)	N.S.
NERVES			
Peripheral Neuropathy	28 (45)	13 (19)	<0.005
Autonomic Neuropathy	1 (2)	0 (0)	N.S.

N.S.: Not significant Level of significance: $P \leq 0.05$

Table 5.9: Microvascular complications in Type 2 nephropathy patients in comparison to those with non-diabetic renal disease

5.5.2.3. Macrovascular Complications

The only significant difference in macrovascular complications between the groups was that 16% of nephropathy patients in comparison to 4% of patients with non-diabetic renal disease had foot/leg ulcers ($P<0.05$) (Table 5.10).

	Nephropathy n (%)	Non-Diabetic Renal Disease n (%)	*P*
HEART			
Ischaemic Heart Disease	12 (19)	13 (19)	N.S.
Myocardial Infarction	8 (13)	10 (15)	N.S.
BRAIN			
Transient Ischaemic Attack	2 (3)	2 (3)	N.S.
Cerebrovascular Accident	2 (3)	8 (12)	N.S.
VASCULAR			
Peripheral Vascular Disease	19 (31)	16 (23)	N.S.
Ulcers (Leg/Foot)	10 (16)	3 (4)	<0.05
Amputation (One Leg)	2 (3)	2 (3)	N.S.

N.S.: Not significant Level of significance: $P \leq 0.05$

Table 5.10: Macrovascular complications in Type 2 nephropathy patients in comparison to those with non-diabetic renal disease

The majority of both groups had hypertension. However, only 42% of the nephropathy group and 52% of those with non-diabetic renal disease with hypertension were being treated with anti-hypertensive medication at referral (Table 5.11). Both groups had systolic hypertension and there was no significant difference in either mean systolic or diastolic pressures between the groups.

	Nephropathy n (%)	Non-Diabetic Renal Disease n (%)	*P*
HYPERTENSION	60 (97)	62 (90)	N.S.
ANTI-HYPERTENSIVE THERAPY	25 (42)	32 (52)	N.S.
SYSTOLIC PRESSURE (mm Hg)			
Range	120-220	110-260	
Mean (±SD)	170(±22)	168(±28)	N.S.
DIASTOLIC PRESSURE (mm Hg)			
Range	50-150	64-120	
Mean (±SD)	90(±15)	90(±12)	N.S.

N.S.: Not significant Level of significance: $P \leq 0.05$ SD: Standard deviation

Table 5.11: Blood pressures in Type 2 nephropathy patients in comparison to those with non-diabetic renal disease

There was a significant difference between the groups in patients who defaulted from routine diabetic clinic appointments: 30 (48%) nephropathy patients versus 13 (19%) in patients with non-diabetic renal disease ($P<0.001$). Defaulting from routine clinic visits increased the risk of developing nephropathy (odds ratio = 4.1). However, there was no difference in smoking history (52% nephropathy and 51% non-diabetic renal disease). Of those patients who had defaulted, 50% with nephropathy and 21% with non-diabetic renal disease had smoked at some time (P=N.S.).

5.5.2.4. Progression of Renal Disease

Forty-two percent of the nephropathy group and 25% of non-diabetic renal disease patients received RRT or died from ESRF. Forty-six percent of nephropathy patients on RRT had CAPD, 54% had haemodialysis treatment and two received kidney transplants while 67% of the non-diabetic renal disease group received CAPD and 33% had haemodialysis, none received renal transplants (*P*=N.S.). Sixteen (26%) nephropathy patients died from ESRF in comparison to two patients with non-diabetic renal disease (*P*=N.S.).

5.6 Discussion

This study has shown that there was a significantly higher proportion of Indo-Asian and Black patients with nephropathy in the Type 2 group. The Southall and Coventry studies have demonstrated that Indo-Asians living in the United Kingdom have an increased incidence of Type 2 diabetes in comparison to the indigenous population [approximately four times greater] (Mather and Keen, 1985; Simmons *et al*, 1989). There is also an increasing body of evidence that Indo-Asian diabetic patients have a higher risk of developing nephropathy and end stage renal disease than White patients (Allawai *et al*, 1988; Burden *et al*, 1992; Feehally, 1995). It has also been demonstrated that Black Type 2 diabetic patients in the United States have an increased incidence of end stage renal disease [up to four times greater] in comparison to White patients (Cowie *et al*, 1989; Perneger *et al*, 1994).

The shorter duration of diabetes at referral and onset of proteinuria in Type 2 patients in this study contrasts with the result of a study in patients who eventually received dialysis where the duration of diabetes in both Type 1 and Type 2 patients was 18 and 16 years respectively (Biesenbach *et al*, 1994). However, the United Kingdom Prospective Diabetes Study in newly diagnosed Type 2 patients has demonstrated that within 9 years of diagnosis 9% of patients had developed microvascular complications despite attempts to normalise glycaemic control within a research protocol (Turner *et al*, 1996). In this study the shorter duration in Type 2 patients until onset of

retinopathy, proteinuria and hypertension contrasts with the longer duration in Type 1 patients and reflects the undetermined duration of diabetes before diagnosis. Many Type 2 patients are unaware that they have the symptoms of diabetes for some time before diagnosis is made; a finding confirmed in the United Kingdom Prospective Diabetes Study where 2% of newly diagnosed Type 2 patients had microvascular complications (Jackson *et al*, 1991; United Kingdom Prospective Diabetes Study Group, 1994).

The strong association between times of onset of retinopathy and especially hypertension and time to development of proteinuria in Type 2 patients is reflective of the progression of microvascular complications and the increase in blood pressure that occurs as nephropathy progresses from microalbuminuria to proteinuria (Mathiesen *et al*, 1995). The longer duration of diabetes to development of proteinuria and accompanying complications in Type 1 patients is consistent with previous research (Parving *et al*, 1988). The possibility that Type 2 patients have accelerated development of complications is probably the result of their older age group and pre-existing vascular damage than to a major difference in the two groups of patients.

The lack of difference between the two groups in prevalence of micro- and macrovascular complications is concerning. As the Type 2 patients were selected for presence of retinopathy in order to remove any query about diagnosis of nephropathy, the similarities in microvascular complications were expected. The concerning aspect is that despite the younger age of the Type 1 patients, the prevalence of macrovascular complications was similar

between the two groups. It has been suggested that Type 1 nephropathy patients have an eight times higher incidence of coronary heart disease in comparison to Type 1 patients without nephropathy (Deckert *et al*,1992).

The positive benefits of anti-hypertensive therapy on the progression of nephropathy have been known for 17 years in Type 1 patients and more recently in Type 2 patients (Mogensen, 1982; Parving *et al*, 1983; Ahmad *et al*, 1997). Despite this, over 90% of patients in both groups had hypertension and less than 50% of these patients were receiving anti-hypertensive medication. However, a recent study in over 3000 Type 1 patients has demonstrated that untreated hypertension is common in people with diabetes (Collada-Mesa *et al*, 1999). Similarly, a German study of pre-dialysis diabetic patients has demonstrated that 97% had poorly controlled blood pressure levels (Pommer *et al*, 1997). In this study, systolic hypertension was common in both groups while diastolic pressures were near the upper limit of normal. This is not unique to patients in Wolverhampton; in France, a high proportion of hypertensive Type 2 nephropathy patients have systolic hypertension despite receiving anti-hypertensive treatment (Passa, 1997).

Over eight years, progression of renal disease to requirement for RRT occurred in a higher proportion of Type 1 patients: 34% in comparison to 19% of the Type 2 group which is interesting in that a larger proportion of the Type 1 group (85%) had serum creatinine concentrations at referral below 300 μmol l^{-1} while only 20% of Type 2 patients had serum creatinine

concentrations at referral above 300 μmol l^{-1} (similar to the percentage who required RRT). These results can be compared with those of Ritz and co-workers who have found that the progression to ESRF in Type 1 and Type 2 patients is the same and that more than 50% of patients in both groups reach ESRF within five years of persistent proteinuria (Ritz *et al*, 1991). However, a small study (n=7 patients) by Friedman and Gross has shown that the decline in renal function is slower in Type 2 than in Type 1 patients, a finding which reflects the results of this study (Friedman and Gross, 1991).

The comparison of Type 2 patients with diabetic nephropathy and those with non-diabetic renal disease has highlighted a number of differences between these two groups. There were more Indo-Asian and Black patients with nephropathy; in contrast, it has been reported that Indo-Asian people in the UK have an higher incidence of non-diabetic renal disease than White patients, and in the USA, Black patients have a seven times increase in odds ratio for end stage renal disease from any cause than White diabetic patients (Perneger *et al*, 1994; Feehally, 1995). The nephropathy patients had a longer duration of diabetes and there were more men than women in comparison to the group with non-diabetic renal disease; findings which are consistent with the study by Perneger and co-workers, 1994.

As expected this study has demonstrated a higher prevalence of retinopathy in the nephropathy group than in those patients with non-diabetic renal disease. A study in India in Type 2 patients has shown that the absence of retinopathy was a more reliable predictor of non-diabetic renal disease in

comparison to the presence of retinopathy in nephropathy patients; due to the variation in the prevalence of retinopathy in Type 2 nephropathy patients, the authors found that retinopathy was a less reliable predictor (John *et al*, 1994). In contrast, in the USA, only 50% of patients with non-diabetic renal disease had retinopathy requiring laser treatment in comparison to 75% of patients with diabetic nephropathy (Perneger *et al*, 1994). In the same study the proportion of patients with peripheral neuropathy was similar in both groups unlike the present study where the nephropathy patients had significantly more peripheral neuropathy than patients with non-diabetic renal disease (Perneger *et al*, 1994).

When a comparison of the time to development of retinopathy, hypertension and proteinuria was made between patients with nephropathy and those with non-diabetic renal disease, there was a shorter time to development of complications in the non-diabetic renal disease group, in keeping with the shorter duration of diabetes at referral, and a stronger correlation between time to development of retinopathy and proteinuria than in the nephropathy patients. A possible explanation for this is that there were only 16 patients with non-diabetic renal disease who had retinopathy in comparison to 63 nephropathy patients; a study with larger numbers of patients with non-diabetic renal disease may produce more conclusive results. The two distinct groups of patients with onset of hypertension pre- and post-diagnosis of diabetes was not surprising as a large percentage of patients had renal disease secondary to hypertension. This may also explain why more patients

with non-diabetic renal disease with hypertension were receiving anti-hypertensive treatment.

A higher percentage of nephropathy patients required RRT and died of ESRF over the eight year period suggesting that the decline in renal function was more rapid in the nephropathy group. However, these results contrast with a Spanish study in which 53% of the patients had non-diabetic renal disease and were followed for less than three years, where there was no difference in the decline of renal function between patients with nephropathy and those with non-diabetic renal disease (Wagner *et al*, 1997).

5.7 Conclusions

1 Type 2 nephropathy patients were older and appeared to have a shorter duration of diabetes at nephrological referral and before onset of proteinuria, retinopathy and hypertension than Type 1 patients; however, this was probably due to the presence of diabetes for an undetermined period of time before diagnosis in Type 2 patients rather than to a real difference between the two groups.

2 There were more Indo-Asian and Black patients with nephropathy in the Type 2 group than in the Type 1 group.

3 There was a stronger relationship between the time to development of retinopathy, hypertension and proteinuria in the Type 2 nephropathy patients than in the Type 1 group.

4 There was no difference between Type 1 and Type 2 nephropathy patients in prevalence of either microvascular or macrovascular complications.

5 There were fewer Indo-Asian and Black Type 2 patients with non-diabetic renal disease and fewer on insulin treatment than Type 2 nephropathy patients.

6 Duration of diabetes at referral and at onset of proteinuria were shorter in patients with non-diabetic renal disease than in the nephropathy group.

7 There was a stronger relationship between time to onset of proteinuria and hypertension in the Type 2 nephropathy group than in patients with non-diabetic renal disease.

8 The nephropathy group had more retinopathy, peripheral neuropathy and leg/foot ulcers than patients with non-diabetic renal disease.

9 The decline in renal function was faster in the nephropathy group than in patients with non-diabetic renal disease.

10 Defaulting from routine clinic visits increased the risk of developing nephropathy.

5.7.1 Summary of Conclusions

1 The prevalence of complications were similar in Type 1 and Type 2 nephropathy patients. However, the relationship between the onset of complications was stronger in the Type 2 patients than in the Type 1 nephropathy group. Therefore, there were similarities and differences between the groups and both $H_0(2)$: Diabetic complications were similar in Type 1 and Type 2 patients with nephropathy and $H_1(2)$:

Diabetic complications were different in Type 1 and Type 2 patients with nephropathy, could be accepted.

2 The prevalence of complications and the relationships between the complications were different in Type 2 nephropathy patients in comparison to Type 2 patients with non-diabetic renal disease. Therefore, accept $H_1(3)$: Diabetic complications were different in Type 2 nephropathy patients compared to Type 2 patients with non-diabetic renal disease.

3 The decline in renal function was faster in Type 2 patients with nephropathy in comparison to Type 2 patients with non-diabetic renal disease. Therefore, accept $H_1(4)$: The progress of renal disease in patients with nephropathy was faster than that of patients with non-diabetic renal disease.

Chapter 6

Comparison of Type 1 Diabetic Patients With and Without Nephropathy

6.1. Introduction

Many studies have been performed to ascertain the factors that put Type 1 diabetic patients at risk of developing nephropathy. The Diabetes Control of Complications Trial has demonstrated that improved glycaemic control reduces the development of microvascular complications in these patients (Diabetes Control and Complications Trial, 1993). The control of hypertension in Type 1 patients with proteinuria has long been recognised as a means of delaying the progression of nephropathy (Mogensen, 1982). Since then, many anti-hypertensive strategies have been used to find the most effective treatment in these patients (Parving *et al*, 1983; Bjorck *et al*, 1986). Microalbuminuria can be reduced and even delayed by the use of angiotensin converting enzyme inhibitors (ACEI) (Viberti *et al*, 1994; EUCLID Study Group, 1997). However, hypertension is still a major problem when patients have proteinuria.

This study was performed with the aim of identifying factors in our Type 1 patients that can be used to differentiate those at risk of developing nephropathy from patients of similar diabetes duration who do not develop nephropathy.

6.2. Objectives

1 To identify differences in diabetes control and diabetic complications between Type 1 diabetic patients with and without nephropathy.

2 To identify risk factors for the development of nephropathy in Type 1 patients.

6.3. Hypotheses

1 Ho: There are no differences in diabetes control and diabetic complications between the groups.

H_1: There are differences in diabetes control and diabetic complications between the groups.

2 Ho: There are no factors other than poor glycaemic control, hypertension and duration of diabetes that put patients at risk of developing nephropathy that can be identified during routine clinical practice.

H_1: There are factors other than poor glycaemic control, hypertension and duration of diabetes that put patients at risk of developing nephropathy that can be identified during routine clinical practice.

6.4 Methods

Using retrospective data, a controlled, matched comparison was made between 23 Type 1 White patients with nephropathy and serum creatinine levels <200 μmol l^{-1} who were referred for nephrological assessment and a control group of 73 Type 1 White patients with no proteinuria and creatinine levels within the normal limits (60-120 μmol l^{-1}) who attended the diabetic clinic for routine annual review over a three year period between 1992-1995. The control group was matched for age, duration of diabetes and gender by identifying White patients whose age and duration of diabetes lay within ten year bands, to match with nephropathy patients, from a cohort of over 200 patients. The same type of data was collected as in Chapter 4: information on demographics, diabetes history, treatment, diabetic control and complications, and details on renal function. A point prevalence i.e.: prevalence at a single examination, was determined for diabetes complications and other factors which may be predictive of development of nephropathy.

All glycated haemoglobin (HbA1) results over a four-year period were collected from medical records and comparisons were made between the groups as well as a separate comparison of HbA1 results at either nephrological assessment or annual review examination (controls).

Comparisons were made of clinical parameters (age, duration of diabetes, BMI, HbA1, diastolic and systolic blood pressures) in patients with pharmacologically treated and untreated hypertension, both between and within the groups.

Descriptive statistics (means $\pm$standard deviation), Student t tests, Chi^2 tests, Mann-Whitney U tests, Pearson correlation, linear and logistic regression analysis were performed using SPSS and Excel statistics packages. The chosen level of statistical significance was $P \leq 0.05$ in two-tailed tests.

6.5 Results

From the original cohort of 49 Type 1 patients referred for nephrological assessment, 23 White patients with nephropathy were compared with 73 matched White controls with no evidence of renal disease on "Albustix" testing (routine testing for microalbuminuria was not performed within the diabetic clinic, therefore no information was available on microalbuminuric status of the control group). The age and duration of diabetes were closely matched. However, the range of duration in both groups was wide (34 versus 40 years) (Table 6.1).

	Nephropathy n (%)	Controls n (%)	*P*
Total number	23	73	
Male	17 (74)	50 (68)	N.S.
Female	6 (26)	23 (32)	N.S.
Age (years)			
Range	21-63	20-68	
Mean (±SD)	40 (12)	43 (12)	N.S.
Median	40	42	
Duration DM (years)			
Range	4-38	7-47	
Mean (±SD)	22 (9)	23 (8)	N.S.
Median	25	20	

D.M.: Diabetes Mellitus S.D.: Standard deviation Level of significance: $P \leq 0.05$

Table 6.1: Demography of Type 1 patients with nephropathy in comparison to matched controls.

Glycated haemoglobin (HbA1) at referral was very much higher in the nephropathy group: mean (±SD): 12.1 (±2.0) % in comparison to 10.7 (±1.9) % in the controls (P<0.005). When comparisons were made of HbA1 over a four year period the control group had much better glycaemic control: mean (±SD): 10.7 (±2.3) % versus 12.3(±2.6) % in the nephropathy group (P<0.001). Analysis of variance demonstrated that there were no statistical differences in HbA1 over the four years within either of the groups (Figure 6.1).

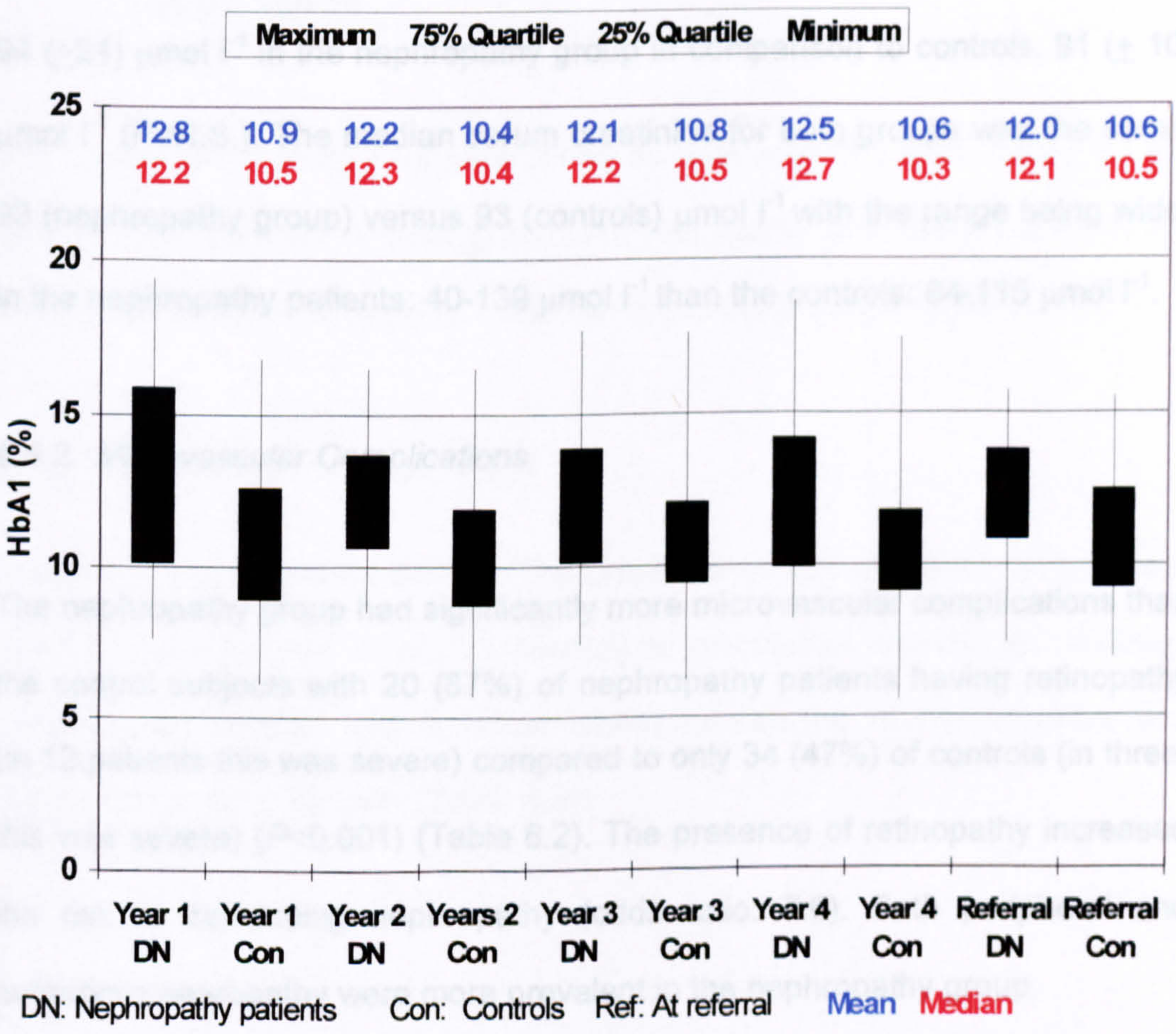


Figure 6.1:Glycaemic control in Type 1 nephropathy patients in comparison to matched controls

The range of body mass index (BMI) was similar in both groups: 21.4-34.3 Kg m^{-2} in nephropathy patients and: 19.9-32.3 Kg m^{-2} in the controls. However, the nephropathy patients were heavier with the differences between the means being statistically significant: 27.9 (±3.6) Kg m^{-2} (nephropathy group) and 25.8(±3.1) Kg m^{-2} (control group) (P<0.05).

6.5.1 *Renal Function*

Both groups had a mean serum creatinine within normal limits (60-120 μmol l^{-1}) 94 (±21) μmol l^{-1} in the nephropathy group in comparison to controls: 91 (± 10) μmol l^{-1} (P=N.S.). The median serum creatinine for both groups was the same: 93 (nephropathy group) versus 93 (controls) μmol l^{-1} with the range being wider in the nephropathy patients: 40-139 μmol l^{-1} than the controls: 64-115 μmol l^{-1}.

6.5.2. Microvascular Complications

The nephropathy group had significantly more microvascular complications than the control subjects with 20 (87%) of nephropathy patients having retinopathy (in 12 patients this was severe) compared to only 34 (47%) of controls (in three, this was severe) (P<0.001) (Table 6.2). The presence of retinopathy increased the risk of developing nephropathy (odds ratio: 7.6). Both peripheral and autonomic neuropathy were more prevalent in the nephropathy group.

Complication	Nephropathy n (%)	Controls n (%)	*P*
EYES			
Retinopathy	20 (87)	34 (47)	<0.001
Severe Retinopathy	12 (60)*	3 (9)*	<0.001
Laser Photocoagulation	12 (60)*	2 (6)*	<0.001
Blind one eye	3 (13)	1 (1)	N.S.
Blind both eyes	0 (0)	1 (1)	N.S.
NERVOUS SYSTEM			
Peripheral Neuropathy	6 (26)	6 (8)	<0.05
Autonomic Neuropathy	2 (9)	0 (0)	N.S.

*: Percentage of those with retinopathy Level of significance: $P \leq 0.05$

Table 6.2: Comparison of microvascular complications: Type 1 nephropathy patients versus controls.

The power of the study was determined as 98% with $P \leq 0.05$, based on the proportion of patients in each group with retinopathy and using the harmonic mean of the number of patients in the two groups (35) (Brant, 1999).

6.5.3 *Macrovascular Complications*

There was little difference between the groups in prevalence of either ischaemic heart disease (angina or ECG evidence) or myocardial infarctions. However, nephropathy patients had a much higher prevalence of hypertension and peripheral vascular disease (Table 6.3).

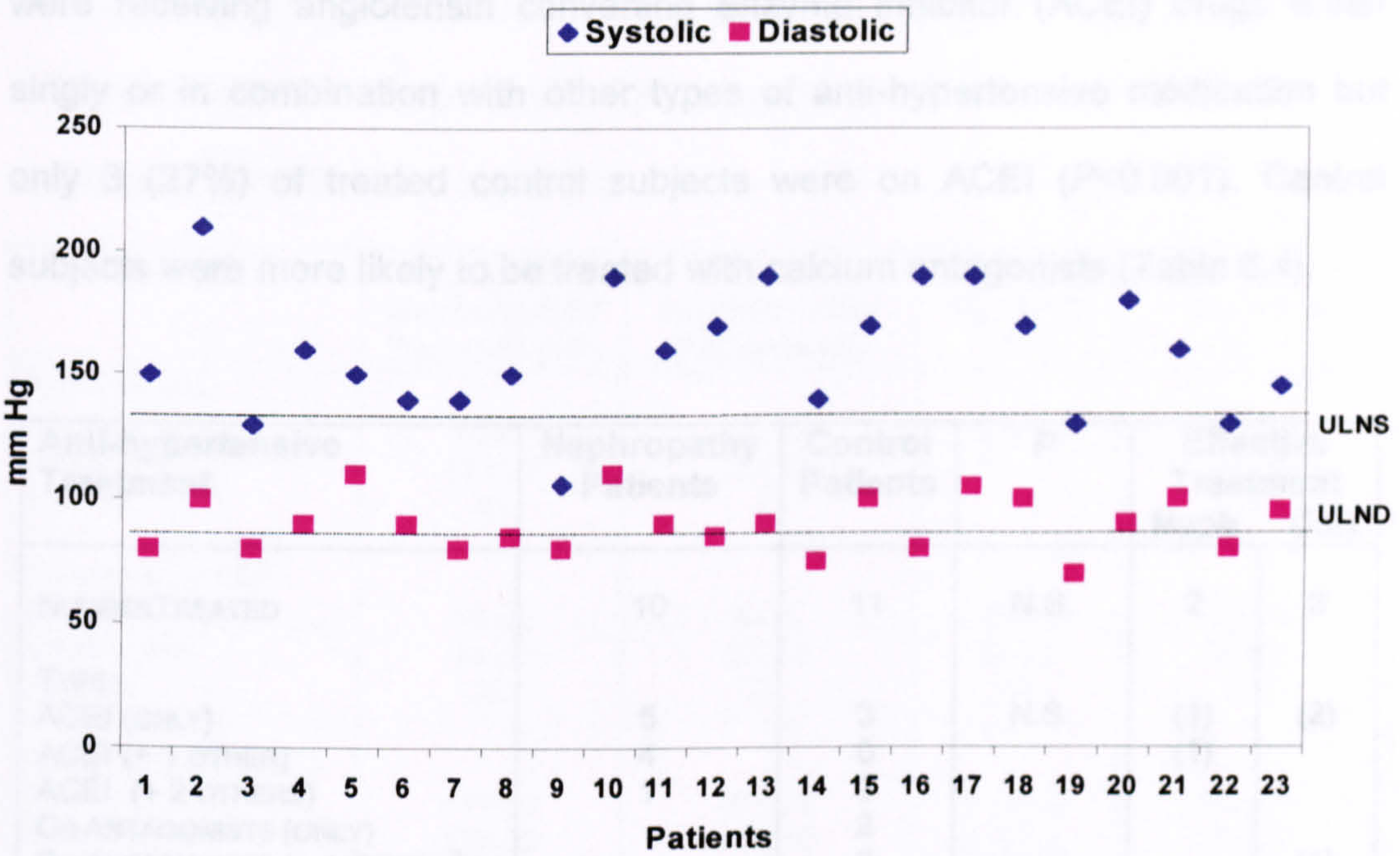
Complication	Nephropathy n (%)	Controls n (%)	*P*
VASCULAR DISEASE			
Peripheral	7 (30)	3 (4)	<0.001
Ulcer	1 (4)	1 (1)	N.S.
BRAIN			
Cerebrovascular Accidents	0 (0)	0 (0)	
HEART			
Ischaemic Heart Disease	2 (9)	4 (5)	N.S.
Myocardial Infarction	3 (13)	3 (4)	N.S.
Hypertension	19 (83)	26 (36)	<0.001

N.S.: Not significant Level of significance: $P \leq 0.05$

Table 6.3: Comparison of macrovascular complications: Type 1 nephropathy patients versus matched controls

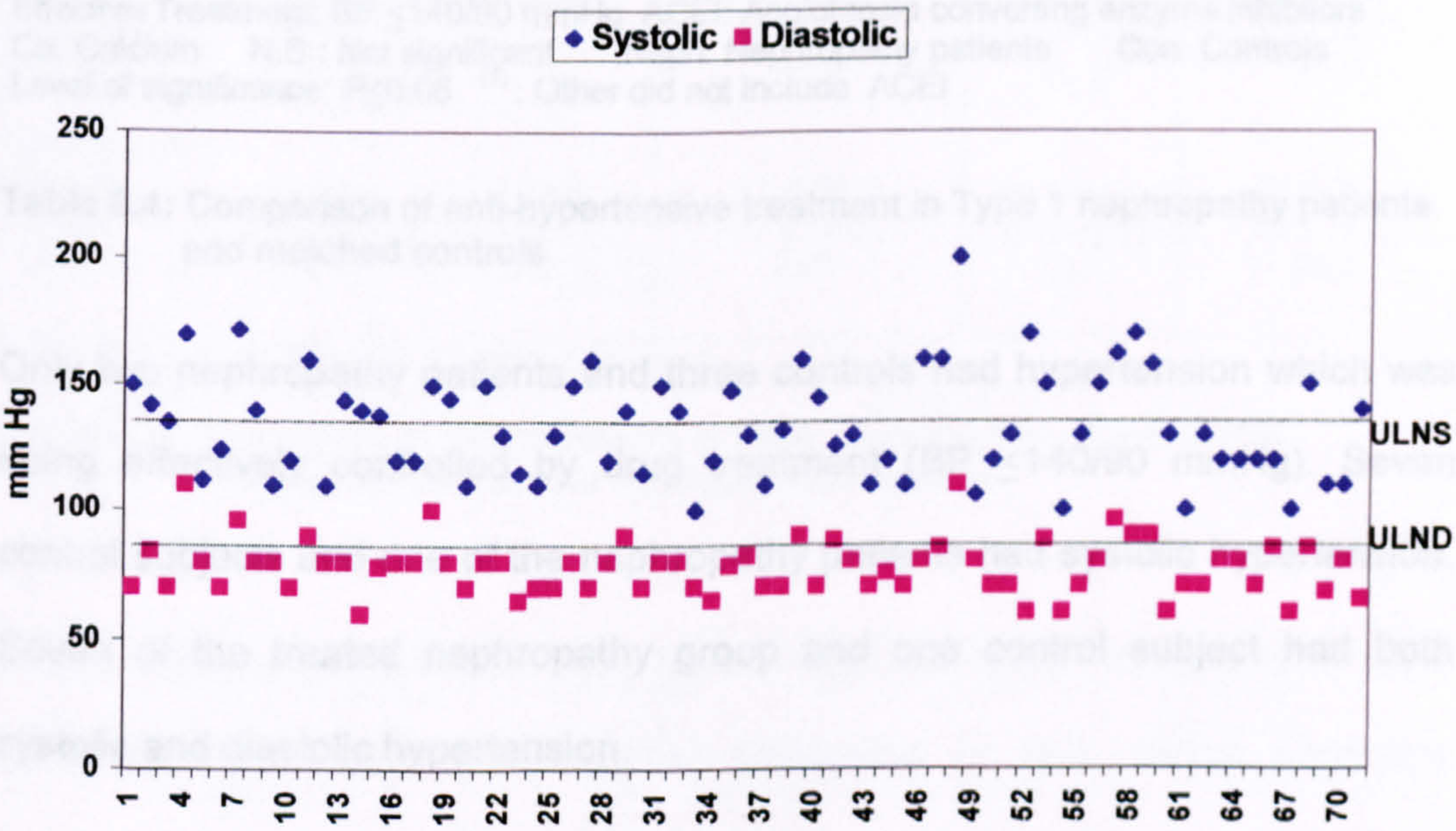
The presence of hypertension increased the risk of developing nephropathy (odds ratio: 8.6). Of those patients with hypertension only 10 (43%) of the nephropathy group and 11 (41%) control subjects were receiving anti-hypertensive medication at referral (*P*=N.S.).

The nephropathy patients had systolic hypertension: mean (±SD): 159(±25)mm Hg versus 134(±21) mm Hg in controls (*P*<0.001), median: 160 (nephropathy group) versus 130 mm Hg (control group). The mean, 90(±11) mm Hg, and median diastolic pressure, 90 mm Hg, in the nephropathy group were at the upper limit of normal in comparison to a mean of 78 (±11) mm Hg and a median of 80 mm Hg in controls (*P*<0.001) (Figures 6.2 and 6.3).



ULNS: Systolic upper limit of normal ULND: Diastolic upper limit of normal

Figure 6.2: Blood pressure in Type 1 nephropathy patients



ULNS: Systolic upper limit of normal ULND: Diastolic upper limit of normal

Figure 6.3: Blood pressure in Type I control group

When the groups were divided into sub-groups with treated hypertension, (10 nephropathy patients versus 11 controls) all of the treated nephropathy patients were receiving angiotensin converting enzyme inhibitor (ACEI) drugs either singly or in combination with other types of anti-hypertensive medication but only 3 (27%) of treated control subjects were on ACEI (P<0.001). Control subjects were more likely to be treated with calcium antagonists (Table 6.4).

Anti-hypertensive Treatment	Nephropathy Patients	Control Patients	*P*	Effective Treatment	
				Neph	Con
NUMBERTREATED	10	11	N.S.	2	3
TYPE:					
ACEI (ONLY)	5	3	N.S.	(1)	(2)
ACEI (+ 1 OTHER)	4	0		(1)	
ACEI (+ 2 OTHERS)	1	0			
Ca ANTAGONISTS (ONLY)		2			
Ca ANTAGONISTS (+ 1 OTHER [1])		2			(1)
BETA BLOCKERS (ONLY)		2			
DIURETICS (ONLY)		2			

Effective Treatment: BP ≤140/90 mmHg ACEI: Angiotensin converting enzyme inhibitors
Ca: Calcium N.S.: Not significant Neph: Nephropathy patients Con: Controls
Level of significance: $P \leq 0.05$ (1) : Other did not include ACEI

Table 6.4: Comparison of anti-hypertensive treatment in Type 1 nephropathy patients and matched controls

Only two nephropathy patients and three controls had hypertension which was being effectively controlled by drug treatment (BP ≤140/90 mmHg). Seven control subjects and one of the nephropathy patients had systolic hypertension. Seven of the treated nephropathy group and one control subject had both systolic and diastolic hypertension.

The treated nephropathy patients were younger: mean age: 41(±13) years in comparison to the control group: mean age: 58 (±7) years and this difference was statistically significant (P<0.005) (Table 6.5). There was no statistical difference between the groups in BMI, creatinine, HbA1 at referral, duration of diabetes or systolic blood pressure

	Treated Nephropathy Patients (10)	Untreated Nephropathy Patients (8)	Treated Controls (11)	Untreated Controls (17)
AGE (YEARS)				
RANGE	24-63	29-62	48-68	24-62
MEAN (±SD)	41 (13)	43 (13)	58 (7)	47 (9)
MEDIAN	41	40	55	45
DURATION OF DM				
RANGE	14-38	8-35	17-41	9-34
MEAN (±SD)	24 (8)	22 (9)	30 (7)	23 (8)
MEDIAN	25	22	30	22
CREATININE (umol l^{-1})				
RANGE	71-139	40-122	71-115	82-114
MEAN (±SD)	99(20)	90 (24)	90 (15)	93 (8)
MEDIAN	95	94	93	93
HbA1 (%)				
RANGE	10.7-13.9	7.4-13.3	8.0-14.3	6.9-13.8
MEAN(±SD)	12.2 (1.3)	11.0 (2.0)	11.2 (2.0)	10.7 (2.1)
MEDIAN	11.7	11.4	11.5	10.5
BMI (kg m^{-2})				
RANGE	27.0-33.4	21.5-34.3	23.7-30.4	22.8-31.2
MEAN	29.8 (2.1)	27.2 (4.3)	27.6 (2.7)	26.1 (2.8)
MEDIAN	29.4	26.1	28.4	25.3

D.M.: Diabetes mellitus SD: Standard deviation

Table 6.5: Comparison of clinical factors in Type 1 nephropathy patients and controls with treated and untreated hypertension.

When nephropathy patients with treated and untreated hypertension were compared there were no statistical differences in age, serum creatinine

concentration, duration of diabetes, HbA1 at referral or systolic pressure (*P*=N.S.). There was a statistical difference in diastolic blood pressure with a higher mean in the treated nephropathy patients (97[±9] mm Hg) in comparison to those with untreated hypertension: (89[±9] mm Hg) (*P*=0.5).

Comparison of nephropathy and control subgroups with treated hypertension demonstrated that the diastolic pressure in nephropathy patients was higher than in the control subjects: 97 (±9) mm Hg versus 81 (±9) mm Hg (*P*<0.005). Both groups had systolic hypertension with a mean systolic pressure in nephropathy patients of 165 (±25) mm Hg in comparison to the control group where the mean was: 149 (±9) mm Hg (*P*=N.S) (Figure 6.4).

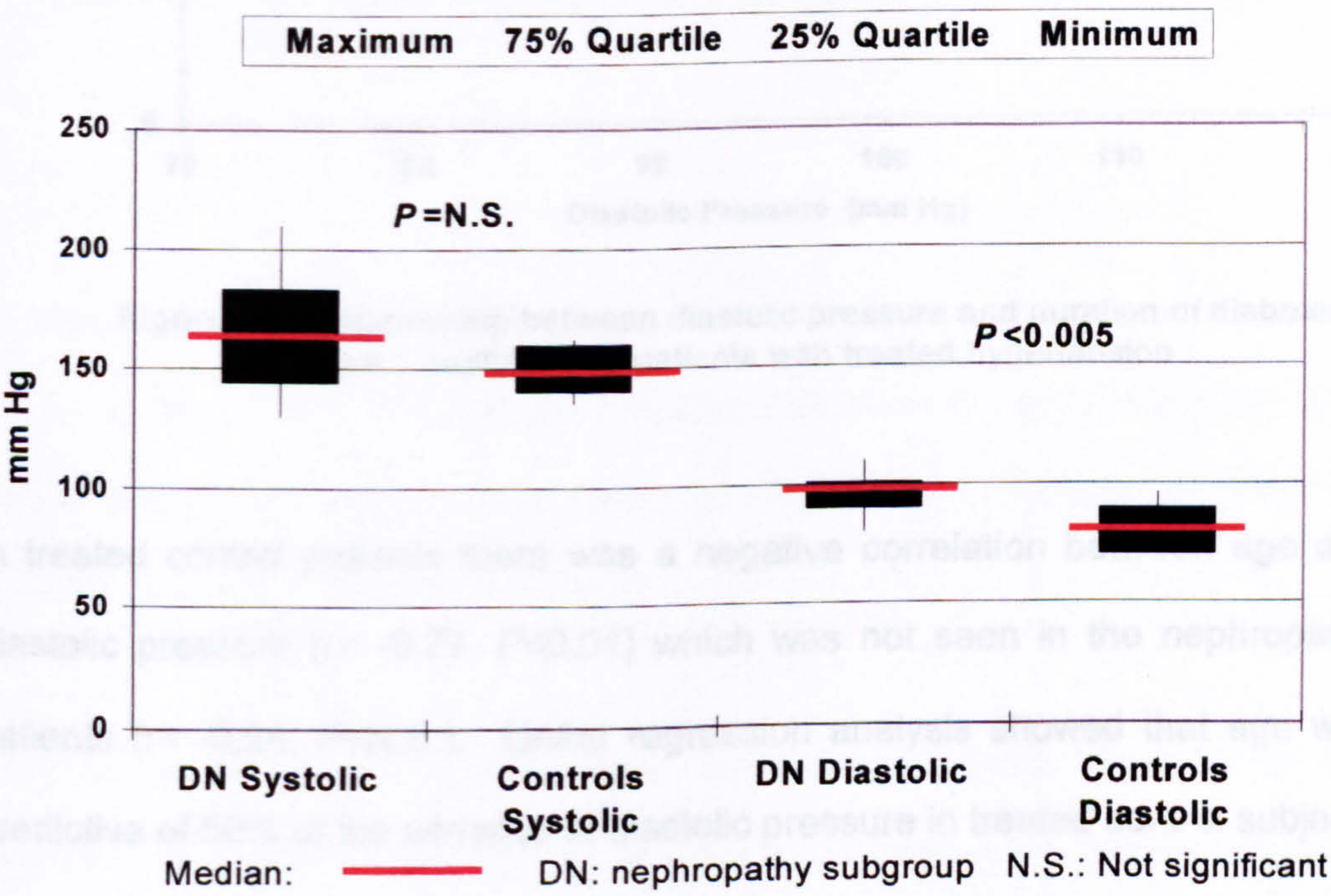


Figure 6.4: Blood pressure in patients with treated hypertension

There was a negative correlation between duration of diabetes and diastolic blood pressure in the treated nephropathy group (r= -0.64, n=10, $P<0.05$) but none in the treated control group (Figure 6.5). Linear regression analysis demonstrated that duration of diabetes was 40% predictive of variance in diastolic pressure in treated nephropathy patients.

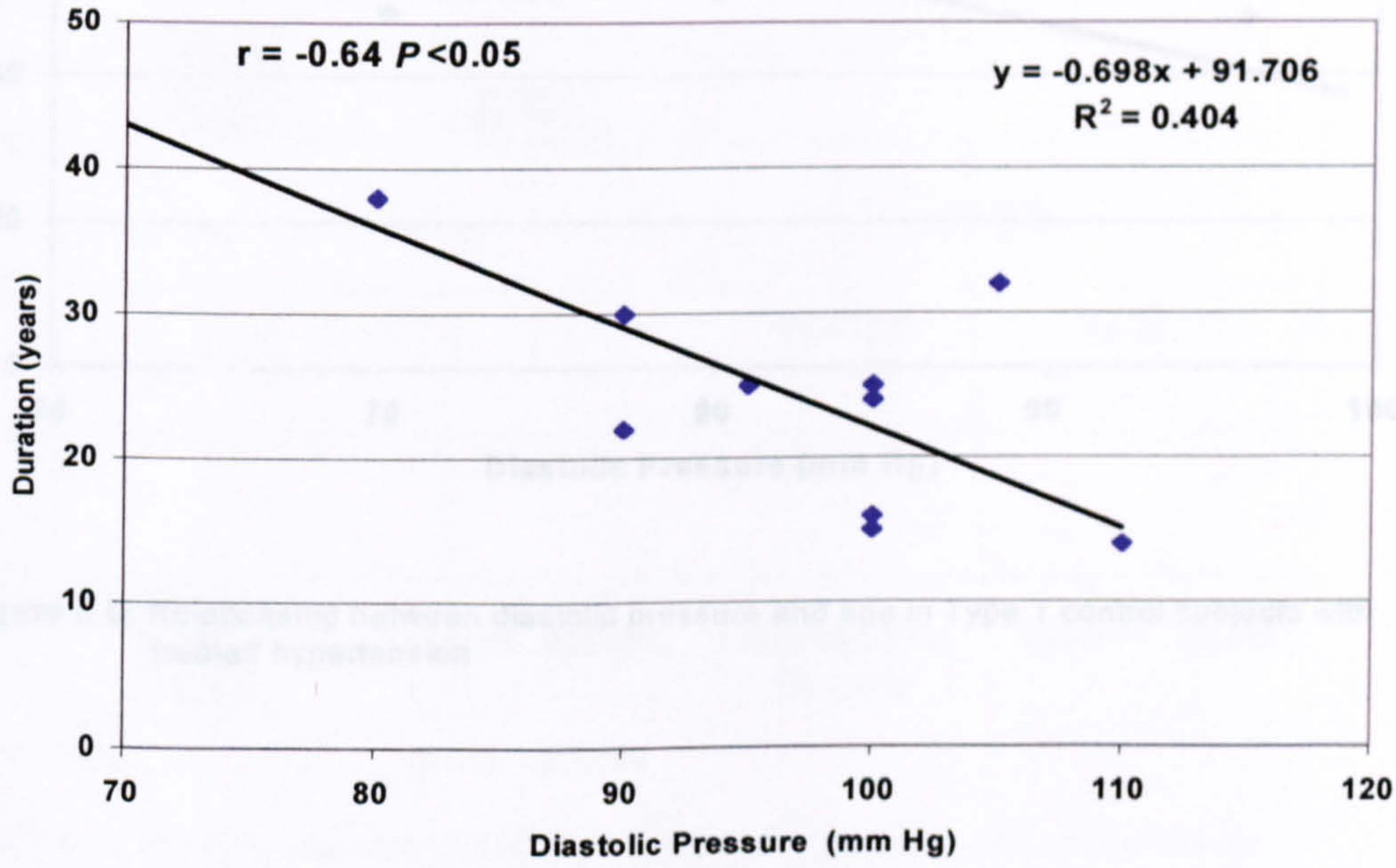


Figure 6.5: Relationship between diastolic pressure and duration of diabetes in Type 1 nephropathy patients with treated hypertension

In treated control patients there was a negative correlation between age and diastolic pressure (r= -0.77, $P<0.01$) which was not seen in the nephropathy patients (r= -0.24, P=N.S.). Linear regression analysis showed that age was predictive of 59% of the variance of diastolic pressure in treated control subjects

(Figure 6.6).

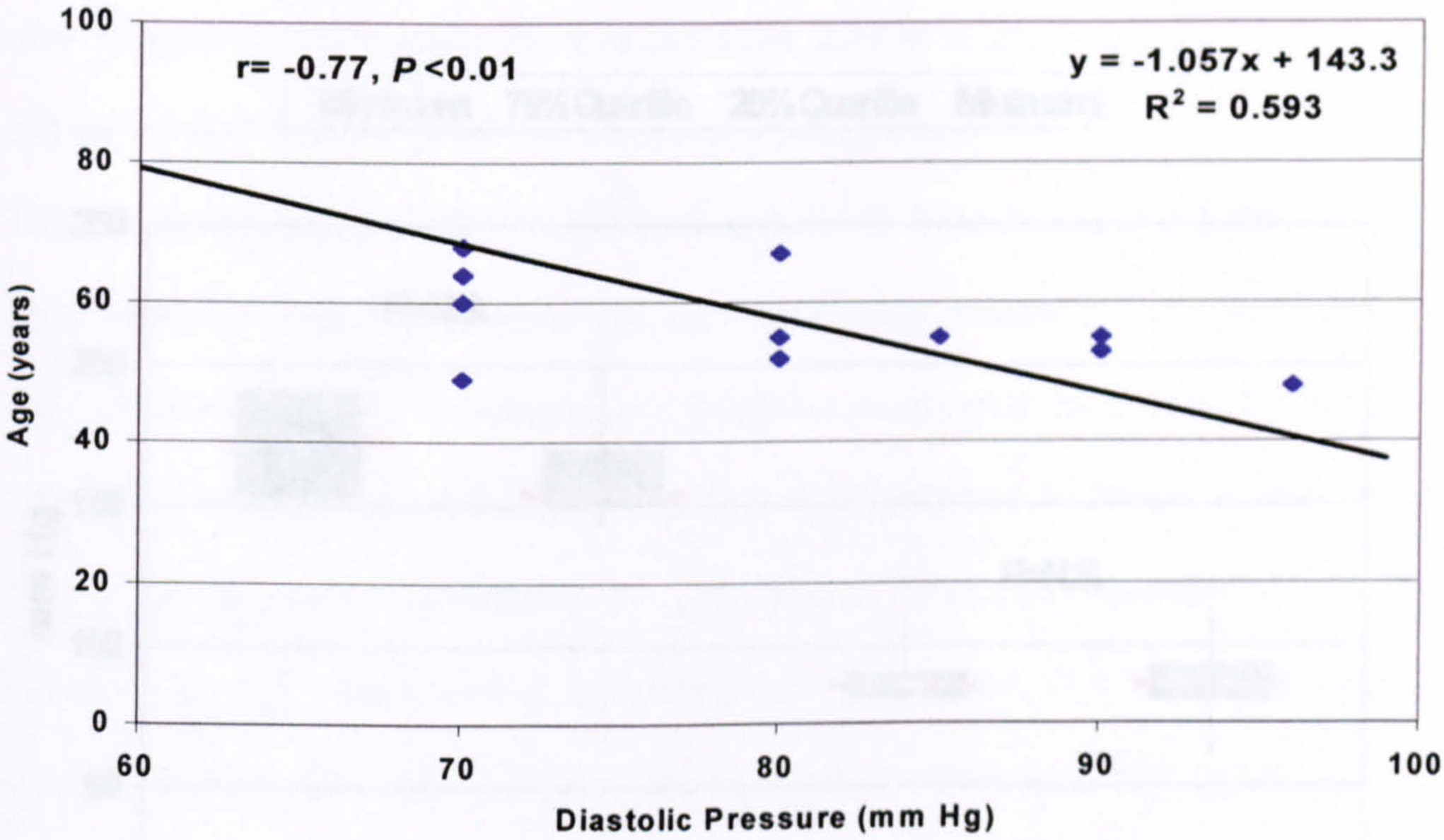


Figure 6.6: Relationship between diastolic pressure and age in Type 1 control subjects with treated hypertension

In patients with untreated hypertension (8 nephropathy patients and 17 control subjects) there were no statistical differences between the groups in mean age, serum creatinine concentration, Hba1 at referral, BMI, duration of diabetes, diastolic or systolic pressures (Table 6.5).

Both untreated groups had systolic hypertension: mean systolic in the nephropathy patients was 170(±18) mm Hg versus 159(±14) mm Hg in control

subjects (*P*=N.S.). Mean diastolic pressures in both groups were below the upper limits of normal: 89 (±9) mm Hg in the nephropathy group in comparison

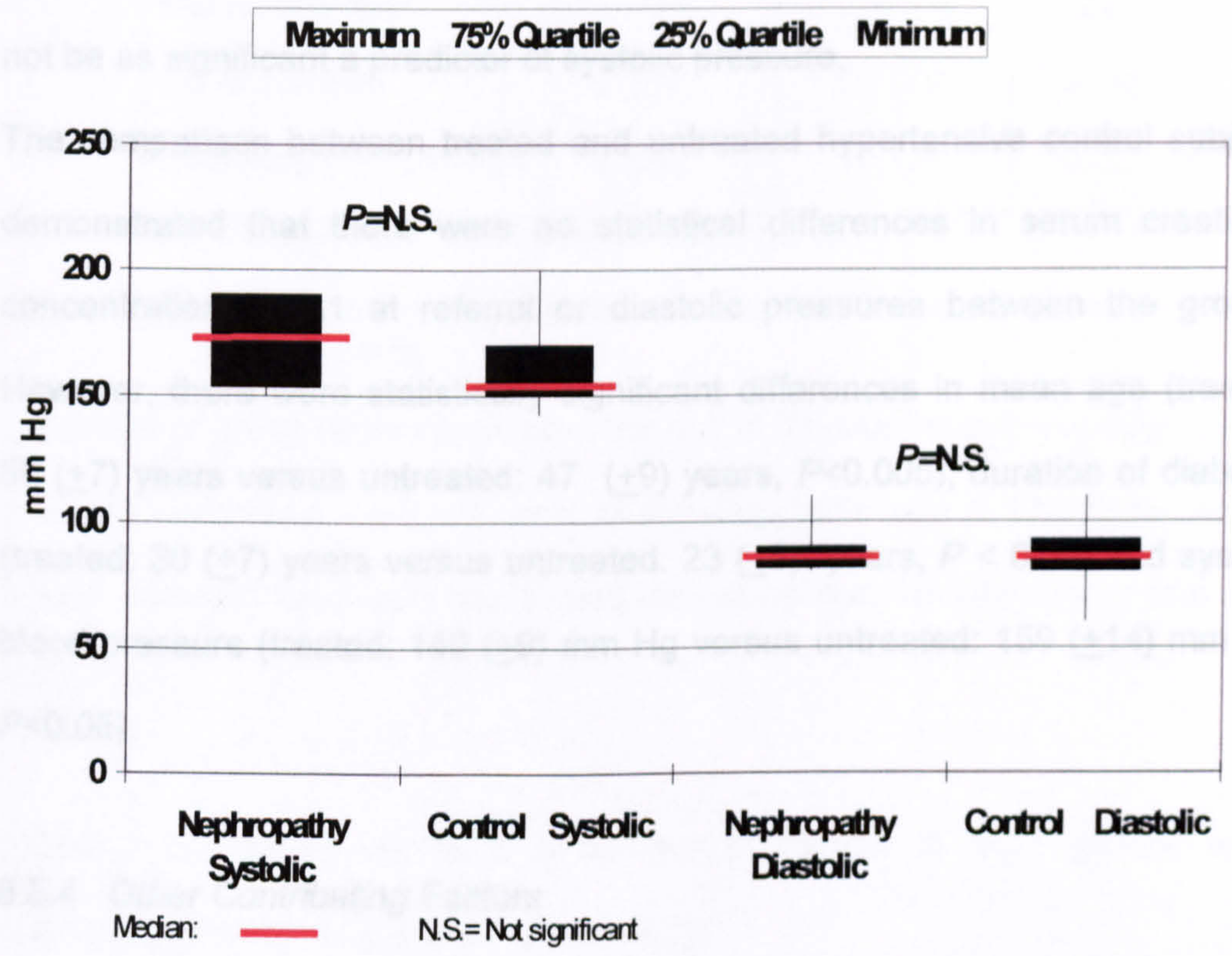


Figure 6.7: Blood pressures in Type 1 nephropathy patients and controls with untreated hypertension

to 87(±13) mm Hg in control subjects (*P*=N.S.). The systolic range in nephropathy patients was wider than in the control group but the diastolic range was narrower than in the control subjects (Figure 6.7).

In the sub-group of untreated nephropathy patients, there was a significant negative correlation between systolic pressure and HbA1 at referral (r= -0.91,

P<0.005). Linear regression analysis showed that HbA1 was 83% predictive of the variance in systolic blood pressure but as the untreated nephropathy group only contained eight patients, it is possible that in a larger group HbA1 would not be as significant a predictor of systolic pressure.

The comparison between treated and untreated hypertensive control subjects demonstrated that there were no statistical differences in serum creatinine concentration, HbA1 at referral or diastolic pressures between the groups. However, there were statistically significant differences in mean age (treated: 58 (±7) years versus untreated: 47 (±9) years, *P*<0.005); duration of diabetes (treated: 30 (±7) years versus untreated: 23 (±8) years, $P < 0.05$) and systolic blood pressure (treated: 149 (±9) mm Hg versus untreated: 159 (±14) mm Hg, *P*<0.05).

6.5.4 *Other Contributing Factors*

A higher prevalence of current smokers and of people who default from routine diabetic clinic visits were found in the nephropathy group and a higher prevalence of patients who had never smoked was found in the control group. The differences between the groups were statistically significant for all of these factors (Table 6.6).

Other Factors	Nephropathy n (%)	Controls n (%)	*P*
Current Smokers	12 (52)	17 (23)	<0.01
History of Smoking	2 (9)	1 (1)	N.S.
Never Smoked	9 (39)	55 (75)	<0.005
Defaulting from Clinic	9 (39)	10 (14)	<0.01

N.S.= Not significant Level of significance: $P \leq 0.05$

Table 6.6: Other factors that may influence development of nephropathy in Type 1 patients.

A number of these factors increased the risk of developing nephropathy: current smoking odds ratio = 4.3; a history of smoking odds ratio = 12.2 (however the actual numbers were very small) and defaulting from routine clinic visits odds ratio = 4.0

Pearson correlation identified a number of factors in both groups which correlated with blood pressure (Table 6.7).

Group	Factors	Correlation R	*P*	R^2
Nephropathy (WG)	BMI and Diastolic Pressure	0.68	<0.005	47%
Nephropathy Treated HT (SG)	Duration DM and Diastolic Pressure	-0.64	<0.05	40%
Nephropathy Untreated HT (SG)	HbA1 and Systolic Pressure	-0.91	<0.005	83%
Controls (WG)	Age and Diastolic Pressure	0.36	<0.005	13%
Controls (WG)	Age and Systolic Pressure	0.41	<0.001	17%

WG: whole group SG: subgroup HT: Hypertension BMI: Body mass index

Table 6.7: Factors which were predictive of blood pressure levels in both groups of patients.

There was a positive correlation between duration of diabetes and serum creatinine levels in the nephropathy group (r=0.48, $P<0.05$) but none in the control group (Figure 6.8). Linear regression analysis showed that duration of diabetes was 19% predictive of the variance in serum creatinine levels in patients who developed nephropathy.

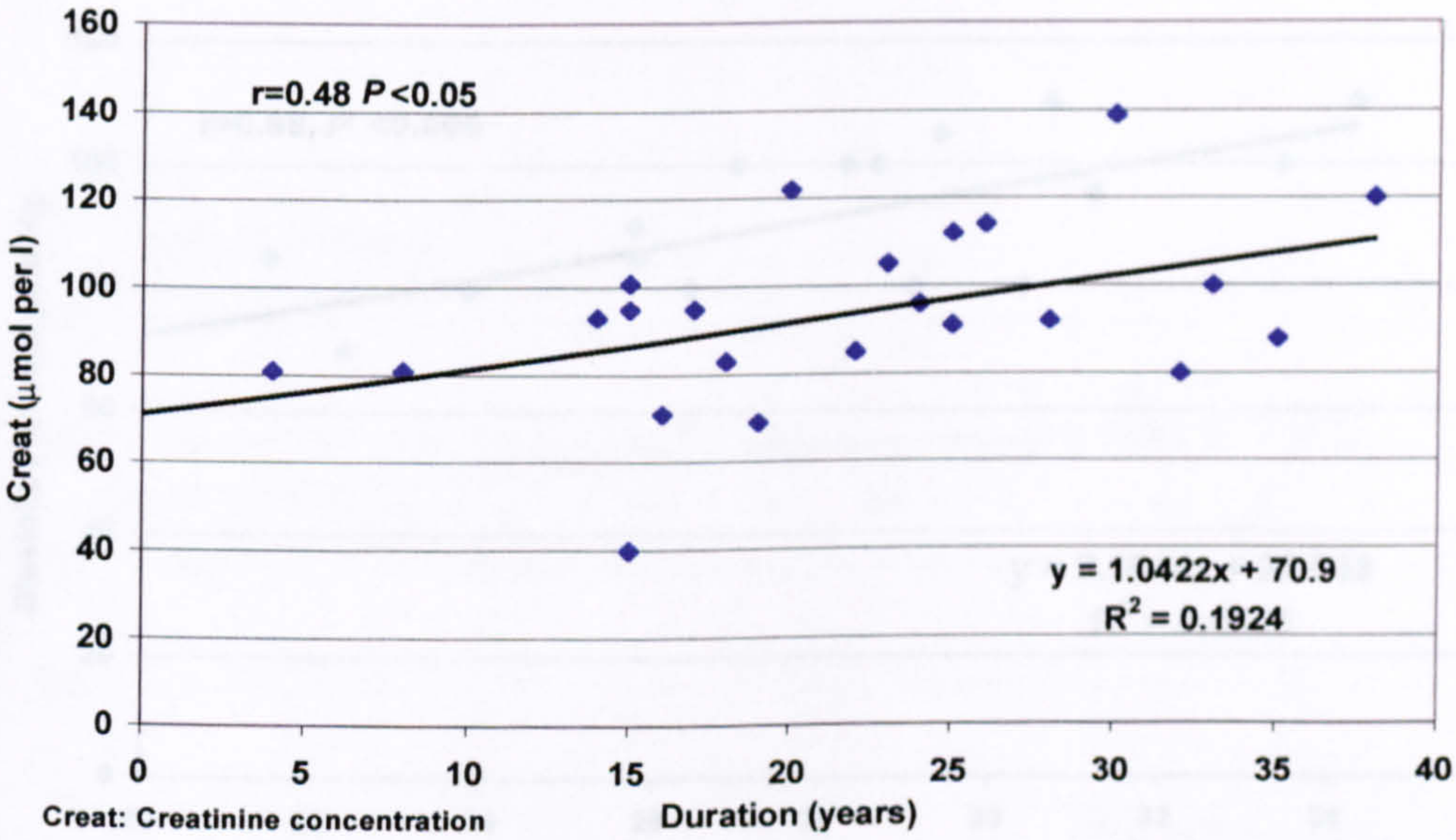


Figure 6.8: Relationship between duration of diabetes and serum creatinine concentration in Type 1 nephropathy patients

Logistic regression analysis using serum creatinine as the dependent variable and defaulting from diabetes clinic visits, hypertension treatment, retinopathy, current smoking, smoking history and gender as independent variables showed

that combined they produced a predictive value of $R^2 = 0.192$ but none of these were statistically significant variables.

The only other factors in the nephropathy group which correlated were body mass index (BMI) and diastolic pressure (r=0.68, *P*<0.005) (Figure 6.9). Linear regression analysis showed that BMI was 47% predictive of the variance in diastolic pressure.

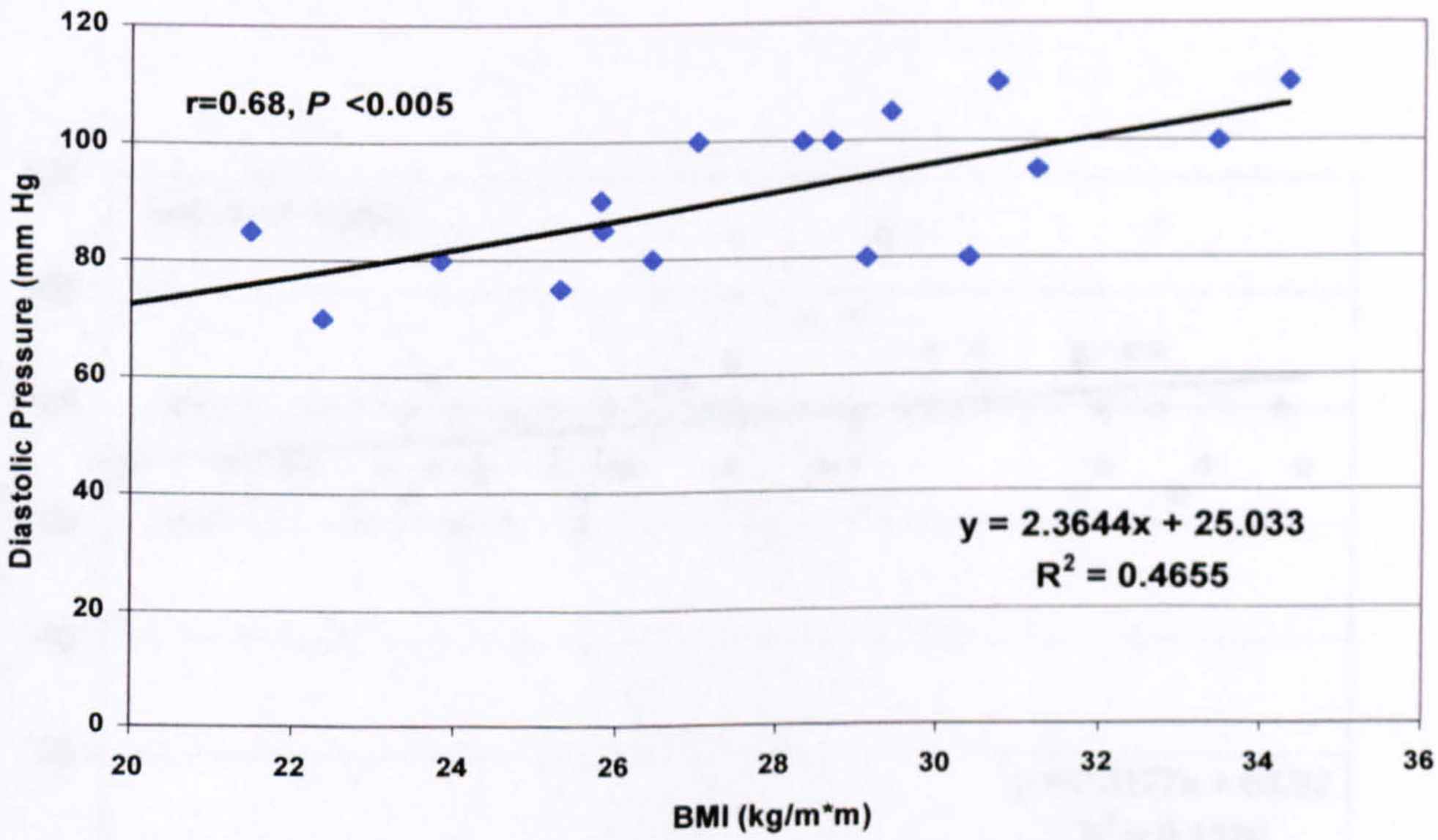


Figure 6.9: Relationship between BMI and diastolic blood pressure in Type 1 nephropathy patients

In the control group as a whole there was a statistically significant positive correlation between age and diastolic pressure (r=0.36, *P*<0.005) in contrast to the negative correlation between age and diastolic pressure in untreated

nephropathy patients (see Figure 6.6) (Figure 6.10).

There was also a positive correlation between age and systolic blood pressure (r=0.41, $P<0.001$) in the control group (Figure 6.11). Regression analysis showed that age was 13% predictive of the variance in diastolic pressure and 17% predictive of the variance in systolic pressure in control subjects.

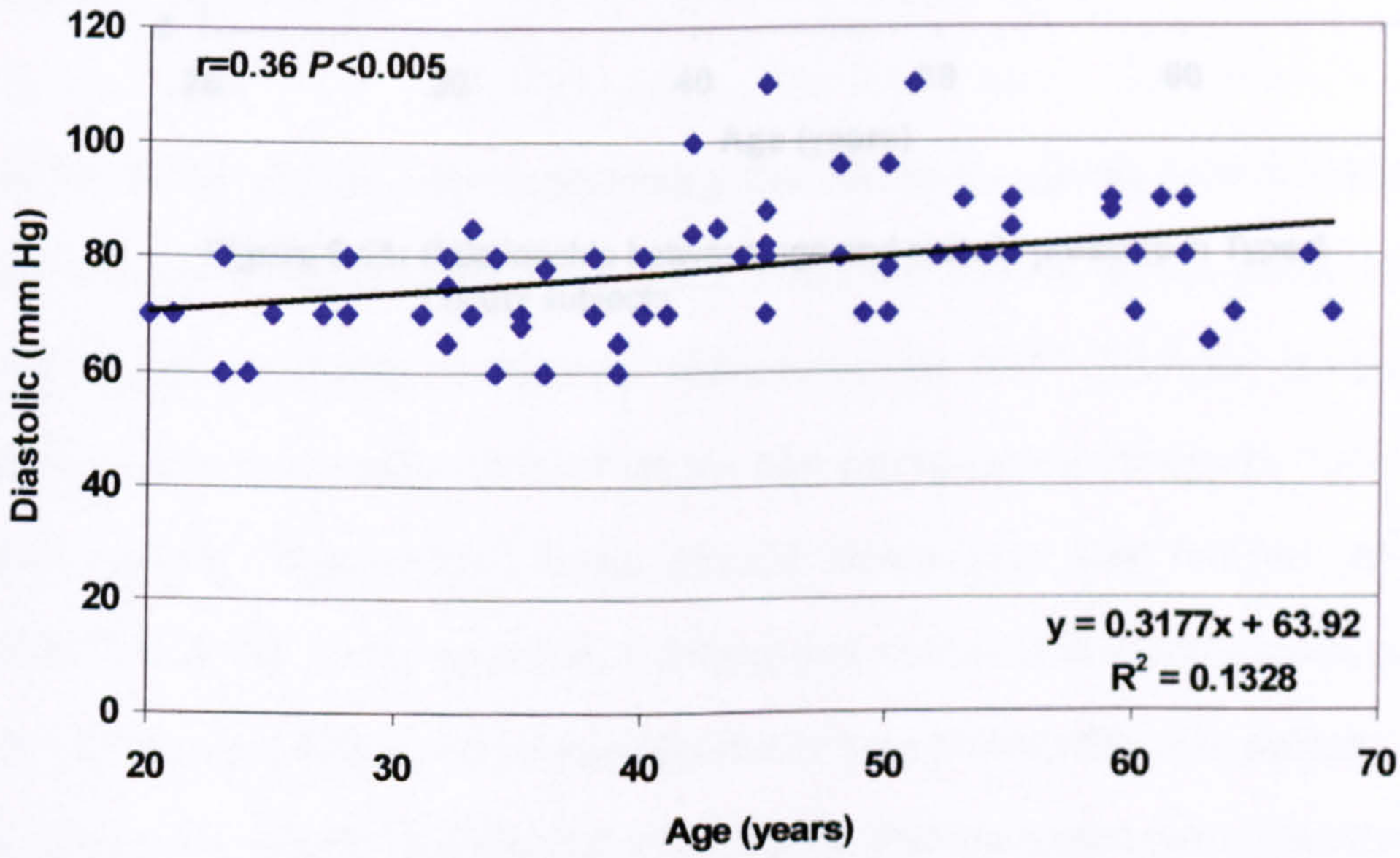


Figure 6.10: Relationship between age and diastolic pressure in Type 1 control subjects

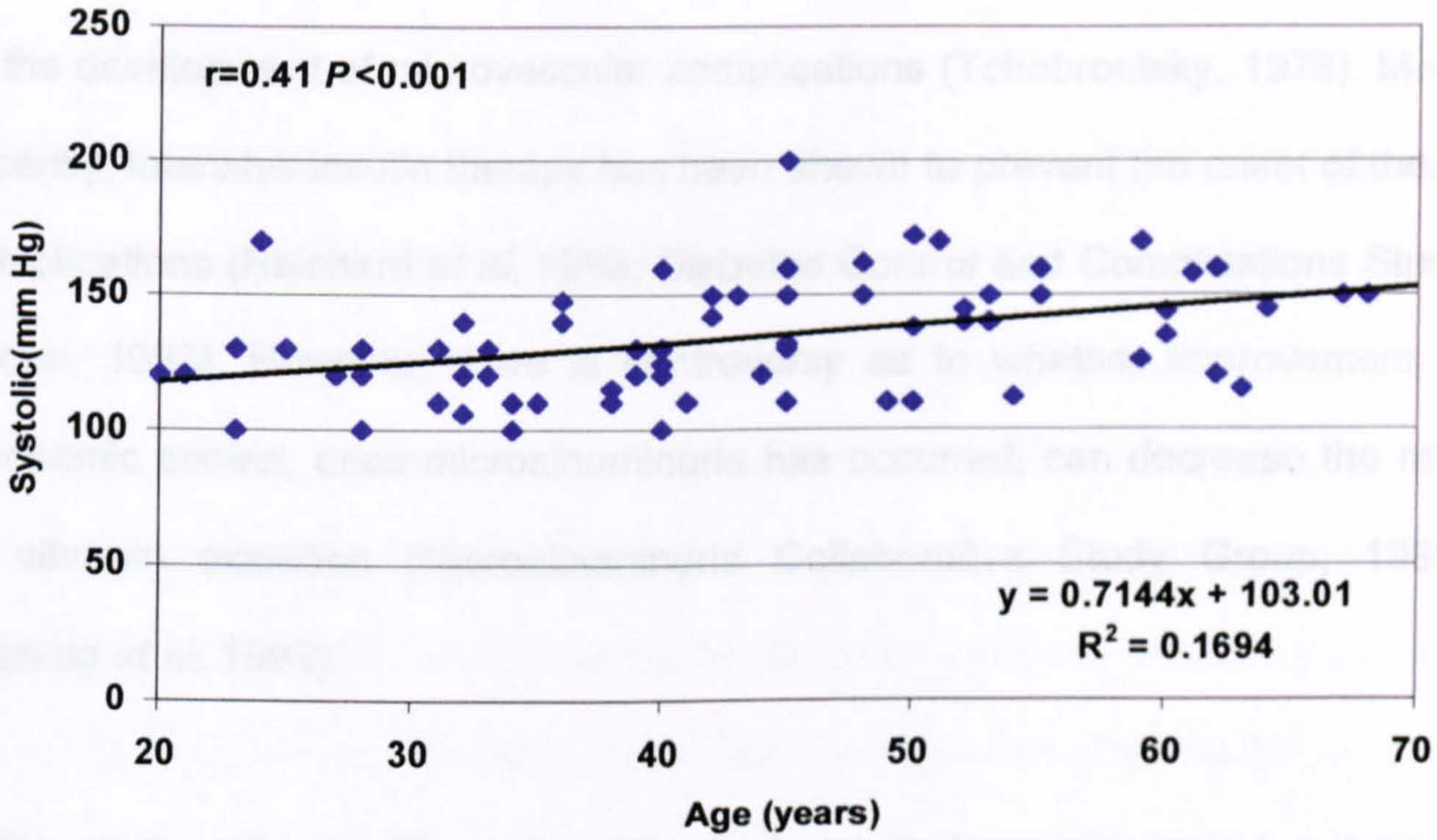


Figure 6.11: Relationship between age and systolic pressure in Type 1 control subjects

6.6 Discussion

It has been known for many years that poor glycaemic control is directly related to the development of microvascular complications (Tchobroutsky, 1978). More recently, intensive insulin therapy has been shown to prevent the onset of these complications (Reichard *et al*, 1993; Diabetes Control and Complications Study Group, 1993). However, there is controversy as to whether improvement in glycaemic control, once microalbuminuria has occurred, can decrease the rate of albumin excretion (Microalbuminuria Collaborative Study Group, 1995; Bojestig *et al*, 1996).

In this study patients with nephropathy had worse glycaemic control and more microvascular complications than the matched control group. Most of the nephropathy patients (87%) had retinopathy (in 60% this was severe) in comparison to controls, 47% of whom had background retinopathy (only 9% had severe retinopathy). These results agree with the findings of the EURODIAB IDDM Complications study where normoalbuminuric patients were found to have background retinopathy rather than proliferative and patients with proteinuria had both (Stephenson *et al*, 1995). The increased risk of developing nephropathy which the presence of retinopathy conferred may be a spurious relationship in that retinopathy develops along with nephropathy and in itself is not necessarily a risk but is indicative of the patient being at risk of developing

renal microvascular disease. In this study nephropathy patients also had more peripheral and autonomic neuropathy, again this is an indication of the extent of microvascular damage.

There was no difference in prevalence of IHD or MI between the groups although the nephropathy group had significantly more PVD and hypertension. The high percentage of patients with hypertension in the nephropathy group was expected as proteinuria and hypertension co-exist in nephropathy. The results for prevalence of hypertension in this study are marginally higher than in the EURODIAB IDDM Complications study, where 69% of macroalbuminuric and 29% of normoalbuminuric patients had hypertension (Collada-Mesa *et al*, 1999). The increased risk which the presence of hypertension confers on patients susceptible to nephropathy is well recognised and the large number of studies on the benefits of controlling hypertension in patients with microalbuminuria testifies to this (Lewis *et al*, 1994; Microalbuminuria Collaborative Study Group, 1995; EUCLID Study Group, 1997). In this study, nephropathy patients had systolic hypertension unlike the control group. Systolic hypertension has been found in previous research in Type 1 nephropathy patients (Cooper *et al*, 1988). Lambert and colleagues have shown that Type 1 patients with microalbuminuria have stiffer carotid arteries in comparison to normoalbuminuric diabetic patients and normal control subjects (Lambert *et al*, 1998). The authors suggest that carotid artery stiffness leads to

systolic hypertension. Arterial rigidity is associated with atherosclerosis and this may explain the significant difference in peripheral vascular disease between the two groups in this study.

It is surprising that less than half of patients with hypertension in both groups (47% versus 48%) were receiving anti-hypertensive medication at examination, especially as many studies have shown the benefit of reducing blood pressure in diabetic patients with nephropathy (Mogensen, 1982; Parving *et al*, 1983; Parving and Hommel, 1989). However, the results of this study are similar to those of the EURODIAB IDDM Complications study where they found that 42.2% of patients with hypertension were receiving anti-hypertensive treatment and that only 11.3% of those patients on treatment had blood pressure levels which were controlled (Collado-Mesa *et al*, 1999). The reasons for the lack of effective anti-hypertensive therapy in both of these studies are likely to be a combination of factors. For example:

- although the recommended upper limits for "normal blood pressure in diabetes" were defined as 140/90 mm Hg by the Working Group on Hypertension in Diabetes in 1987, a treatment threshold of 150/90 or even a systolic pressure above 150 mm Hg (more in line with non-diabetic patients) may have been used in a clinical setting;
- the awareness of medical staff for the need to control blood pressure at "relatively" low levels in patients with proteinuria may be absent or unclear;

- the possibility of side effects from anti-hypertensive drugs (e.g.: impotence secondary to treatment with beta-adrenoceptor blocking drugs) may be regarded by medical staff as unacceptable in relatively young patients (Working Group on Hypertension in Diabetes, 1987).

In the subgroup study comparing patients with treated and untreated hypertension, the numbers of patients in each of the groups were small resulting in a very low statistical power (less than 10%) therefore the sub-study could be regarded more as a pilot study. When patients with treated hypertension in both groups were compared only 20% of the nephropathy group and 27% of control subjects had blood pressures within normal levels i.e.: were receiving effective anti-hypertensive treatment. One nephropathy and seven control patients had systolic hypertension alone, and the rest of the patients in both groups had both diastolic and systolic hypertension.

All of the treated nephropathy patients were receiving ACEI either alone or in combination with other types of anti-hypertensive medication in comparison to only three of the control group. The treated nephropathy group had higher mean diastolic and systolic pressures in comparison to treated control subjects and they were also younger than the controls. The negative correlation between age and diastolic pressure and duration of diabetes and diastolic pressure in the small number of treated nephropathy patients, which was not present in

untreated nephropathy patients, suggests that there may be other factors which separate these two groups other than hypertension treatment, especially as there were no statistical differences in either age or duration of diabetes between the groups. Previous research has suggested that patients with nephropathy have increased total cholesterol, low density lipoprotein, triglyceride and reduced high density lipoprotein concentrations (Jensen *et al*, 1988; Watts *et al*, 1988). This lipid profile would contribute to the development of atherosclerosis even at an early age affecting arterial elasticity and subsequently the blood pressure.

When treated and untreated nephropathy patients were compared the only difference was that those with treated hypertension had higher diastolic blood pressures than those not receiving anti-hypertensive medication. These results, though based on a small sample size, suggest that blood pressure may be more difficult to control in younger nephropathy patients than in control subjects and that there may be two distinct groups of nephropathy patients i.e.: one with more advanced nephropathy (in the treated group mean serum creatinine was 99 μmol l^{-1} versus 90 μmol l^{-1} in untreated patients; with the maximum value in the treated group close to 200 μmol l^{-1}). It may be that ACEI are not as effective in treating hypertension in established nephropathy than in the earlier microalbuminuric stage of the disease. However, the small numbers of nephropathy patients in this study make it impossible to reach definitive

conclusions on this topic and indicate the need for a larger multicentre study to address this.

Two meta-analyses on the effect of anti-hypertensive treatment in nephropathy patients have shown that treatment with ACEI does have a beneficial effect on renal function independently of its effects on blood pressure (Kasiske *et al*, 1993; Maki *et al*, 1995). Early research into the effect of hypertension treatment in nephropathy has demonstrated that reducing blood pressure with a variety of anti-hypertensive agents also reduces the rate of decline of renal function (Parving *et al*, 1983; Mogensen, 1982). More recently, the EUCLID study showed that treatment for 24 months with lisinopril (ACEI) in 34 microalbuminuric (AER 20-200μg min^{-1}) patients, in comparison to 45 microalbuminuric patients receiving placebo, slowed the decline in renal function, although the numbers in both groups were small (EUCLID Study Group, 1997).

In patients with untreated hypertension both the nephropathy and control subjects had systolic hypertension with diastolic pressures within normal limits. This could indicate that systolic hypertension is regarded as a less important parameter in treating hypertension than diastolic pressure despite the evidence that systolic hypertension is a significant factor in the progression of diabetic nephropathy (Cooper *et al*, 1988). In younger untreated control subjects with a

shorter duration of diabetes than treated controls, the mean systolic pressure was significantly higher than that in the treated control subjects. This indicates a need for more intensive monitoring and treatment of systolic hypertension in younger patients.

In this study, the nephropathy group were more likely to have a history of smoking and to default from routine diabetic clinic visits than controls, both of which increased the risk of developing nephropathy. In 1993, Reichard and co-workers found no relationship between smoking and nephropathy in Type 1 patients. However, other researchers have demonstrated a link between the two, (Chase *et al*, 1991; Sawicki *et al* 1994; Aldmal *et al,* 1994). While, strong independent relationships have also been demonstrated between ex-smoking and macroalbuminuria and ex-smoking and proliferative retinopathy (independently of albumin excretion rate) (Chaturvedi *et al*, 1995). Biesenbach and colleagues have also shown that nephropathy patients who smoked not only had an increased rate of decline in renal function but also had higher systolic and diastolic blood pressures than non-smokers (Biesenbach *et al*, 1994). In this study, peripheral vascular disease was more common in the nephropathy patients than controls which may also be related to smoking.

The positive correlation between serum creatinine concentration and duration of diabetes suggested that duration of diabetes was predictive of declining renal

function in the nephropathy patients but not in controls. This may be more reflective of poor glycaemic control over many years of diabetes that has led to the development of nephropathy rather than be directly related to length of duration.

Raised body mass index was predictive of raised diastolic blood pressure in the nephropathy group but again not in control subjects. It is possible that a high body mass index has a contributory effect on diastolic pressure that is already above normal secondary to nephropathy, but in control subjects where blood pressure is not compromised by abnormal renal function, body mass index is far less significant.

In control subjects the positive correlations between age and diastolic pressure and age and systolic pressure were to be expected as blood pressure increases with age in the UK population as a whole.

There may have been an element of bias in the selection of the control subjects as these were people attending for annual review examinations and might have excluded some patients who may have defaulted from clinic visits but who did not have nephropathy. Also, the selection of patients for the control study occurred five years after starting the collection of data in nephropathy patients. In those five years, changes in the approach to treating hypertension and poor

glycaemic control may have occurred which would have been more likely to impact on diabetic people who did not have advanced microvascular disease than on the nephropathy patients.

6.7 Conclusions

1 Nephropathy patients had consistently worse glycaemic control than the control group. Therefore, accept $H_1(1)$: there are differences in glycaemic control between the groups.

2 Nephropathy patients had a higher prevalence of microvascular complications, peripheral vascular disease and hypertension than control subjects. Therefore, accept H_1 (1): There are differences in diabetic complications between the group.

3 The individual presence of retinopathy and / or hypertension both increased the risk of developing nephropathy.

4 Current smoking, a history of smoking and defaulting from routine clinic visits all increase the risk of developing nephropathy. Therefore, accept H_1 (2): there are factors other than poor glycaemic control, hypertension and duration of diabetes that put patients at risk of developing nephropathy that can be identified during routine clinical practice.

Chapter 7

A Comparison of Type 2 Diabetic Patients With and Without Nephropathy

7.1 Introduction

Diabetic nephropathy in Type 1 patients has been studied extensively but until relatively recently the whole topic of nephropathy in Type 2 patients seemed to have little attraction for researchers. The presence of microalbuminuria in Type 2 patients was regarded as predictive of death from cardiovascular disease instead of the early stages of nephropathy (Mogensen, 1984). As management of ischaemic heart disease and hypertension have improved, the prognosis for Type 2 patients with microalbuminuria appears to have improved in terms of survival, and increasingly Type 2 patients are progressing to overt proteinuria and onwards to end stage renal failure. This has stimulated research into the progression of nephropathy in Type 2 patients with microalbuminuria (Berrut *et al*, 1997). Several studies have demonstrated a raised glomerular filtration rate in newly diagnosed Type 2 patients (Vora *et al*, 1993; Ritz and Stefanski, 1996).

Over the last decade people with Type 2 diabetes have increasingly represented a significant number of diabetic patients who receive renal replacement therapy (Humphrey *et al*, 1989; Perneger *et al*, 1994). In different countries the proportion of diabetic patients in end stage renal failure with Type 2 diabetes varies considerably (e.g.: Australia: 61%; New Zealand: 79%; Italy: 67% and Germany: 51%) as does the proportion in different ethnic groups: (data from USA) Black people: 29%, White: 31%; Pima Indians: 95%; Native American Indian: 55%; (data from Australia)

White: 48%; Aboriginal: 83% (Ritz and Stefanski, 1996). This would reflect the incidence of Type 2 diabetes in the respective population groups.

The same clinical problems occur in Type 2 as in Type 1 diabetic patients in terms of preventing microvascular complications and potentially identifying those people who may be at risk of developing nephropathy. This retrospective study has been designed to identify potential risk factors for developing nephropathy in Type 2 patients by direct comparison with a matched control group with normal renal function.

7.2 Objectives

1 To identify differences in diabetic control and complications between Type 2 patients with nephropathy and matched controls.

2 To identify risk factors for the development of nephropathy in Type 2 patients.

7.3 Hypotheses

1 H_0: Type 2 nephropathy patients had the same prevalence of diabetic complications and the same prevalence of hypertension than matched controls.

H_1: Type 2 nephropathy patients had a different prevalence of diabetic complications and a higher prevalence of hypertension than matched controls.

2 H_0: There were no factors other than poor glycaemic control, the presence of hypertension or duration of diabetes that put Type 2 patients at risk of developing nephropathy that can be identified during routine clinical practice.

H_1: There were factors other than poor glycaemic control, the presence of hypertension or duration of diabetes that put Type 2 patients at risk of developing nephropathy that can be identified during routine clinical practice.

7.4 Methods

From the index population of patients referred for nephrological assessment from the diabetic clinic, a group of 46 Type 2 patients with nephropathy and serum creatinine levels less than 300 μmol l^{-1} were compared with 44 matched controls with no evidence of overt proteinuria (routine testing for microalbuminuria was not performed in the diabetic clinic, therefore, microalbuminuric status of control subjects was not known). Nephropathy patients were only included if they had a duration of diabetes greater than two years, had no retinopathy at presentation of diabetes and no proteinuria within the first three years after diagnosis of diabetes. These inclusion criteria were selected in order to identify a discrete group with nephropathy and decrease the likelihood that patients had undiagnosed diabetes for many years before diagnosis. Nephropathy was diagnosed on clinical grounds i.e.: they had to have either proteinuria on "Albustix" testing plus diabetic retinopathy or confirmation of diagnosis by renal biopsy.

The control group was selected from 643 Type 2 patients attending the diabetic clinic for routine annual review examination between 1992-1995. They had normal serum creatinine levels (≤120 μmol l^{-1}) and no evidence of overt proteinuria. Controls were matched for age, duration of diabetes, gender, ethnic group and diabetes treatment where possible.

Data were collected as in Chapter 4 for both groups of patients i.e.: information on diabetes history, treatment and complications, renal disease, hypertension and anti-hypertensive treatment; hypertension was defined as systolic pressure >140 mm Hg and / or diastolic pressure >90 mm Hg. Diabetic control was determined using glycated haemoglobin (HbA1) results at either nephrological assessment or annual review examination (controls). HbA1 results over a four-year period were collected and comparisons were made between the groups. A point prevalence i.e.: prevalence at a single examination, was determined for diabetes complications and other factors that may be predictive of development of nephropathy.

A comparison was made between patients with treated and untreated hypertension in both groups to determine whether there were specific factors which contributed towards lack of anti-hypertensive therapy.

Descriptive statistics (means $\pm$standard deviations), Student t-tests, Chi^2 test and Fisher Exact test when expected frequencies were less than 5 for 2x2 contingency tables, Mann-Whitney U tests for data with abnormal distributions, analysis of variance of between and within the groups was performed for repeated HbA1 measurements over four years, Pearson correlation and linear and multiple regression analysis were performed using SPSS statistics package. Odds ratio was also calculated as a measurement of risk of developing nephropathy. The chosen level of significance was $P \leq 0.05$ for one and two tailed tests.

7.5 Results

The two groups were well matched for age, duration of diabetes, gender and ethnic group. There was a higher percentage of nephropathy patients receiving insulin treatment, but this difference was not statistically significant (Table 7.1).

	Nephropathy n (%)	Controls n (%)	*P*
NUMBERS	46 (100)	44 (100)	N.S.
GENDER			
Male	30 (66)	29 (66)	
Female	16 (34)	15 (34)	N.S
ETHNIC GROUP			
Indo-Asian	15 (29)	13 (30)	
Afro-Caribbean	6 (13)	5 (11)	N.S.
Caucasian	25 (58)	26 (59)	
DIABETES TREATMENT			
Diet	1 (2)	1 (2)	
Oral	9 (22)	18 (41)	N.S.
Insulin	36 (78)	25 (57)	
AGE (Years)			
Range	41-77	42-76	
Mean (±SD)	61(±8)	62(±8)	N.S.
Median	62	63	
DURATION OF DM (Years)			
Range	4-28	4-35	
Mean (±SD)	14(±6)	15(±7)	N.S.
Median	14	14	

DM: Diabetes mellitus N.S.: Not significant Level of significance: $P \leq 0.05$

Table 7.1: Demographics of Type 2 nephropathy patients versus controls

The range in diabetic control (HbA1) at examination was wide in both groups: 6.3-15.5% (controls) and 7.4-20.0% (nephropathy group). There was a statistical difference ($P<0.05$) in mean HbA1 between patients with

nephropathy [11.8 (±2.7)%] and controls [10.4 (±2.0)%]. Analysis of variance of mean HbA1 over four years demonstrated that the nephropathy group had worse control although it was far from ideal in both groups: Mean (±SD) of results for all four years in the nephropathy group: 12.1 (±2.8)% versus 11.3 (±2.4)% in control subjects (P<0.005). There was no statistical difference in mean HbA1 between the 4 years within the nephropathy group but within the control group there was a significant difference in the third year (P<0.05) possibly due to changes in clinical policy (Figure 7. 1).

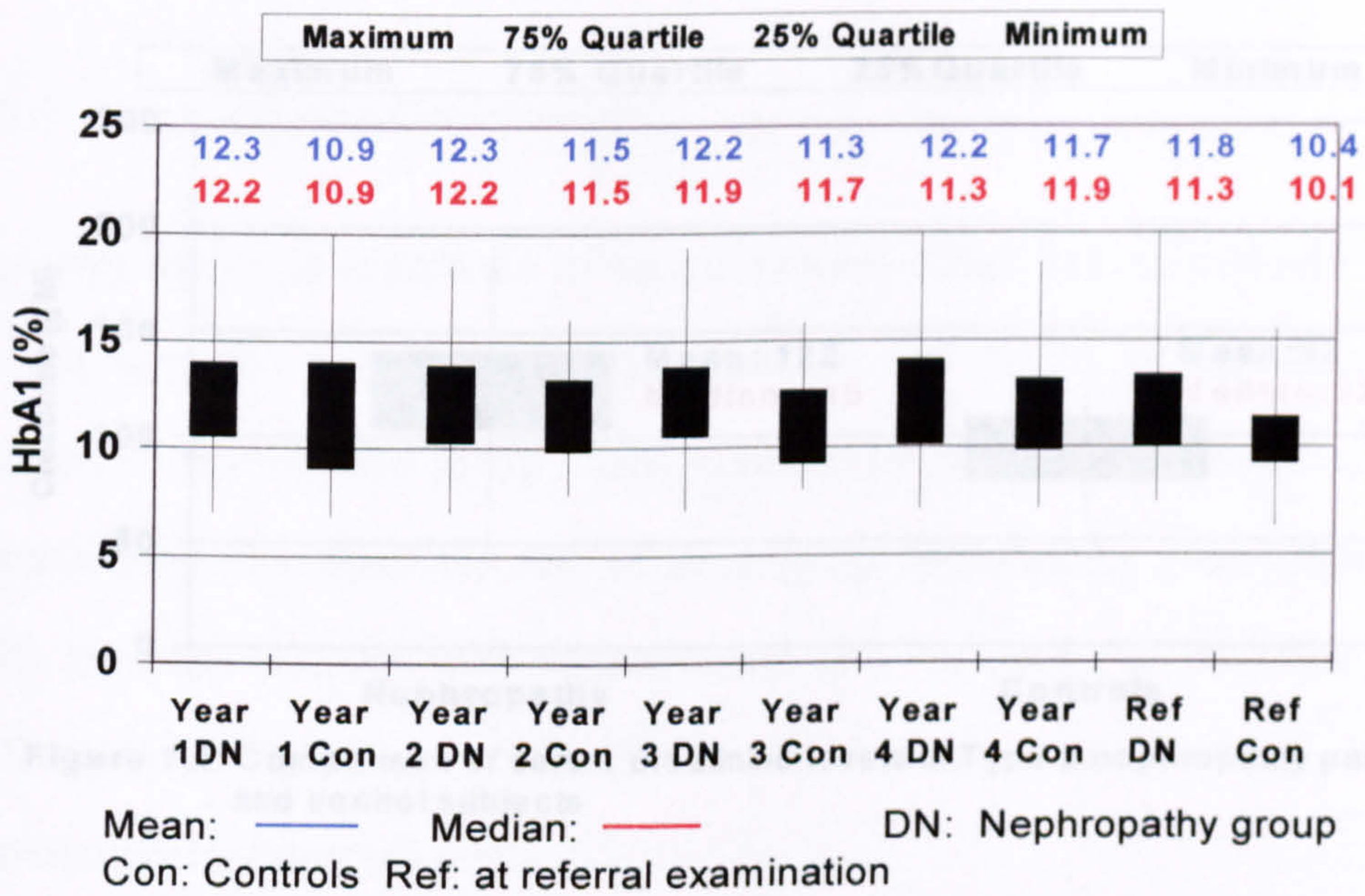


Figure 7.1: Glycaemic control: Type 2 nephropathy patients versus controls

There was no significant difference in body mass index (BMI) between the groups: nephropathy patients: 28.6 (± 2.9) kg m^{-2} versus 27.0 (±5.3) kg m^{-2} (P=N.S.).

7.5.1 Renal Function

The range of serum creatinine in the control group (56-120 μmol l^{-1}) was within the normal reference range (60-120 μmol $l^{-1)}$ with a mean of 92 μmol l^{1}. As expected, the range in the nephropathy group was wider (65-191 μmol l^{-1}), with a mean just above and median within the upper limit of normal (Figure 7.2). The difference between the means was statistically significant (*P*<0.001)

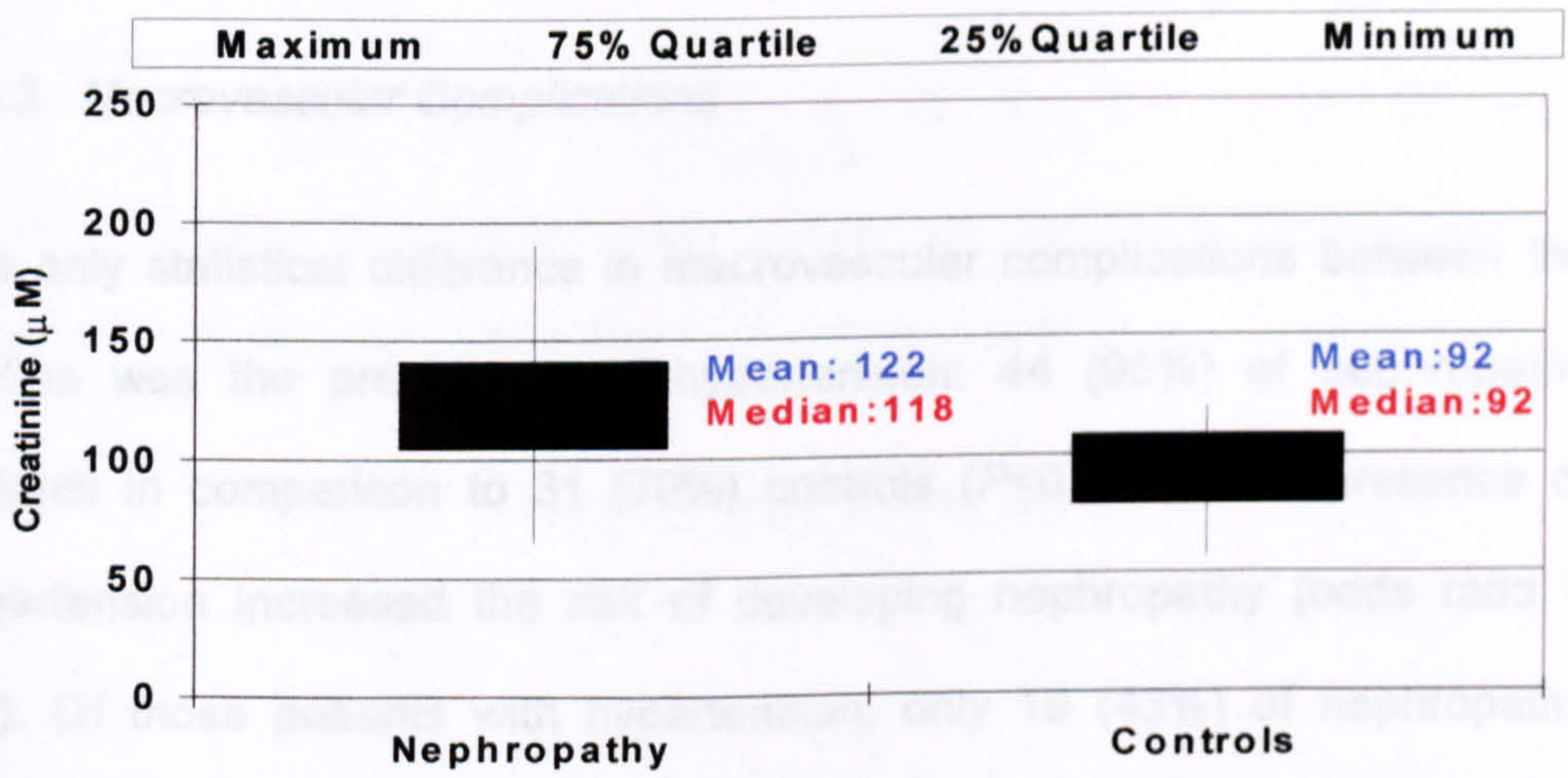


Figure 7.2: Comparison of serum creatinine levels in Type 2 nephropathy patients and control subjects

7.5.2 Microvascular Complications

There was a similar prevalence of microvascular complications in the two groups except for eye complications where there were statistically significant differences between the groups in background retinopathy (*P*<0.001) with the nephropathy patients having more severe retinopathy and treatment with laser photocoagulation (*P*<0.001 for both) (Table 7.2). However, it was to be

expected that nephropathy patients would have retinopathy, as this was part of the clinical criteria for diagnosis of nephropathy.

Eye Complications	Nephropathy n (%)	Controls n (%)	*P*
Retinopathy	43 (93)	20 (45)	<0.001
Severe Retinopathy	24 (56)	1 (5)	N.S.
Laser Photocoagulation	20 (47)	1 (5)	N.S.

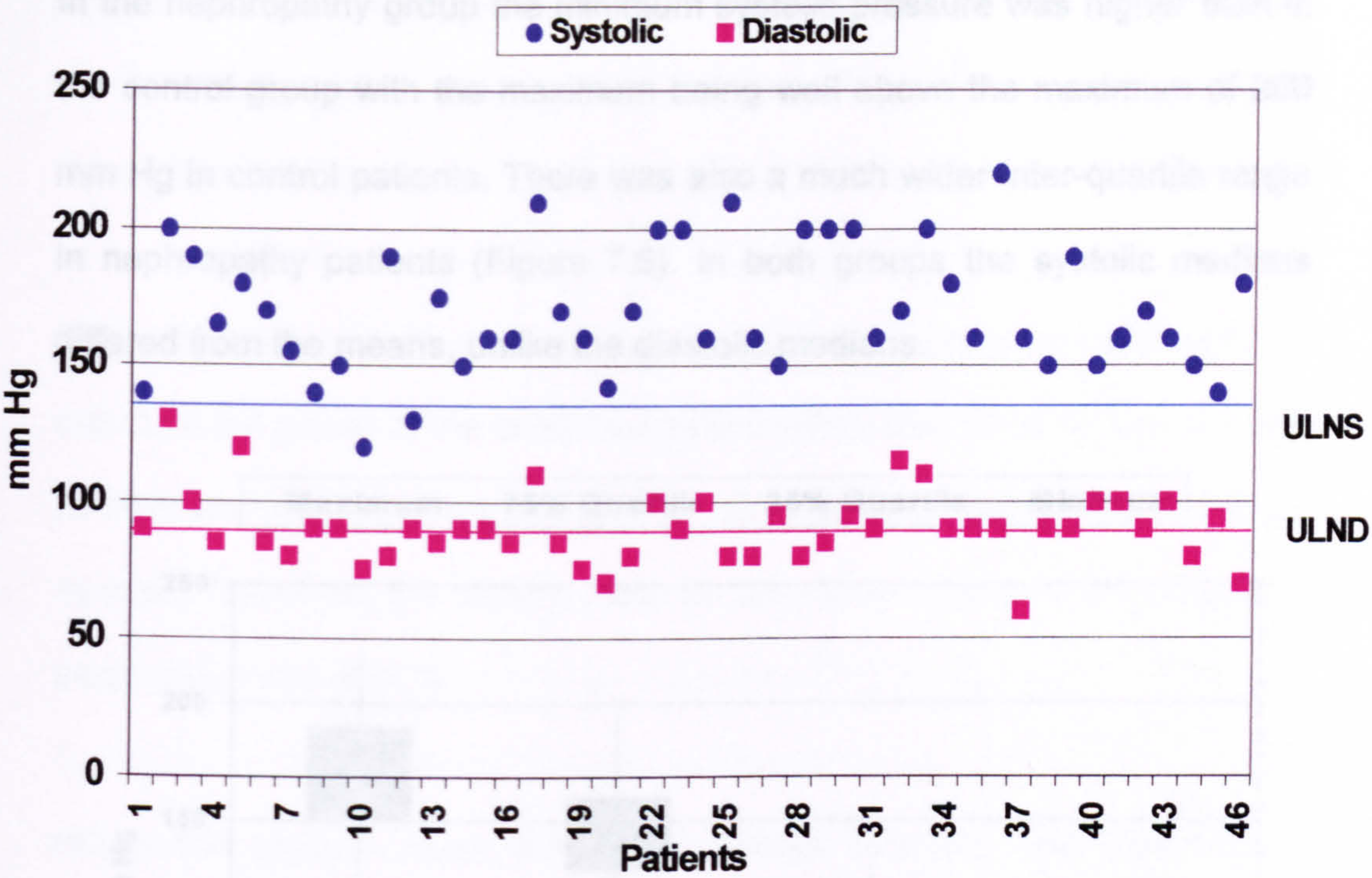
N.S.: Not significant Level of significance: $P \leq 0.05$

Table 7.2: Eye complications in Type 2 nephropathy patients compared to control subjects

7.5.3 Macrovascular Complications

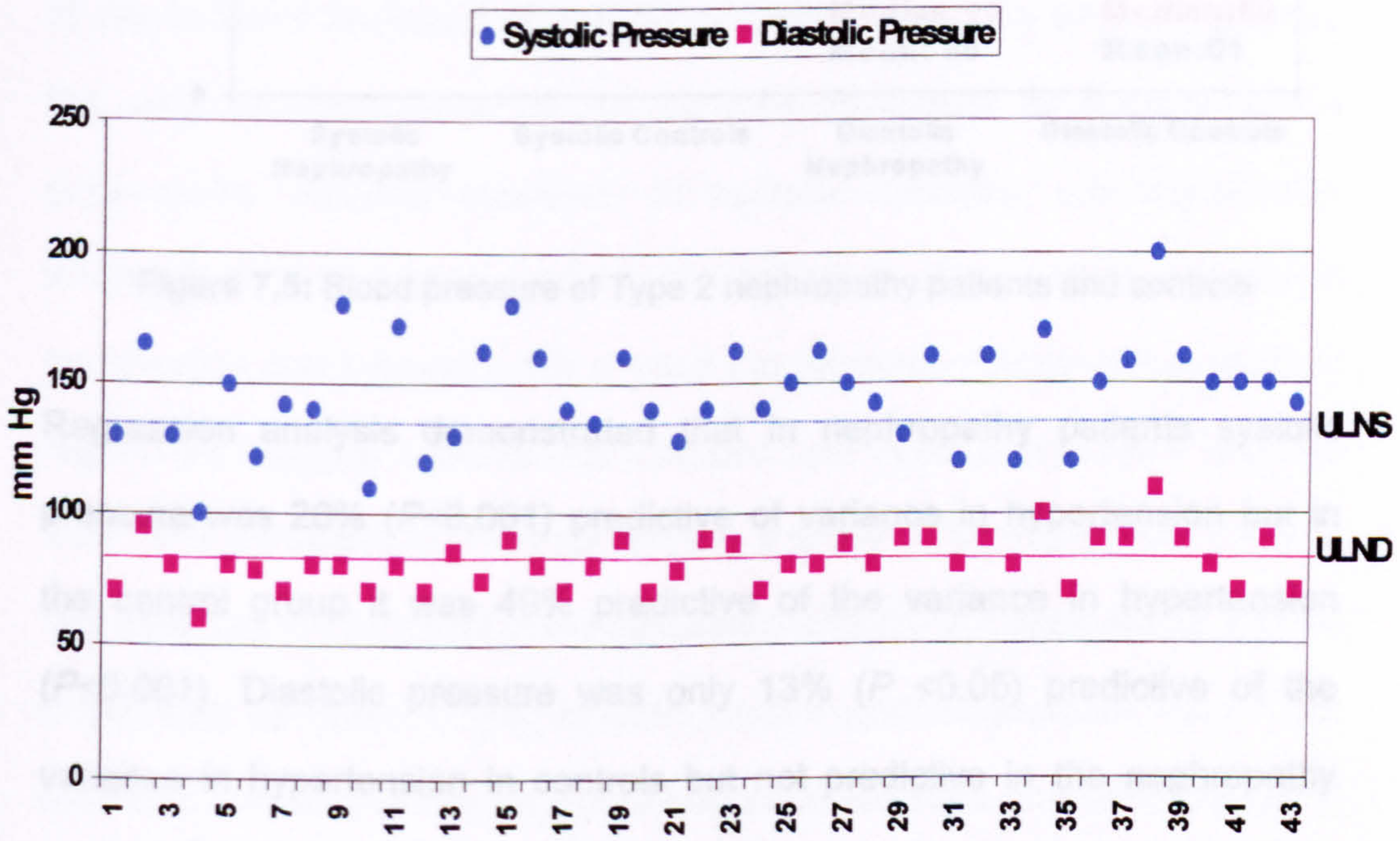
The only statistical difference in macrovascular complications between the groups was the prevalence of hypertension: 44 (96%) of nephropathy patients in comparison to 31 (70%) controls ($P<0.005$). The presence of hypertension increased the risk of developing nephropathy (odds ratio = 9.2). Of those patients with hypertension, only 19 (43%) of nephropathy patients in comparison to 22 (71%) of controls were receiving anti-hypertensive therapy ($P<0.05$).

Both groups had systolic hypertension with diastolic pressures within the normal range. There were statistically significant differences in mean systolic pressures: 170(±23) mm Hg in the nephropathy group versus 146 (±20) mm Hg in the control group ($P<0.001$) and in mean diastolic pressures: 90(±13) mm Hg (nephropathy patients) compared to 81(±10) mm Hg (control subjects) ($P<0.001$) (Figures 7.3 and 7.4).



ULNS: Upper limit of normal Systolic Pressure ULND: Upper limit of normal Diastolic pressure

Figure 7.3: Blood pressures in Type 2 nephropathy patients



ULNS: Upper limit of normal Systolic Pressure ULND: Upper limit of normal Diastolic pressure

Figure 7.4: Blood pressure in Type 2 control subjects

In the nephropathy group the minimum systolic pressure was higher than in the control group with the maximum being well above the maximum of 200 mm Hg in control patients. There was also a much wider inter-quartile range in nephropathy patients (Figure 7.5). In both groups the systolic medians differed from the means, unlike the diastolic medians.

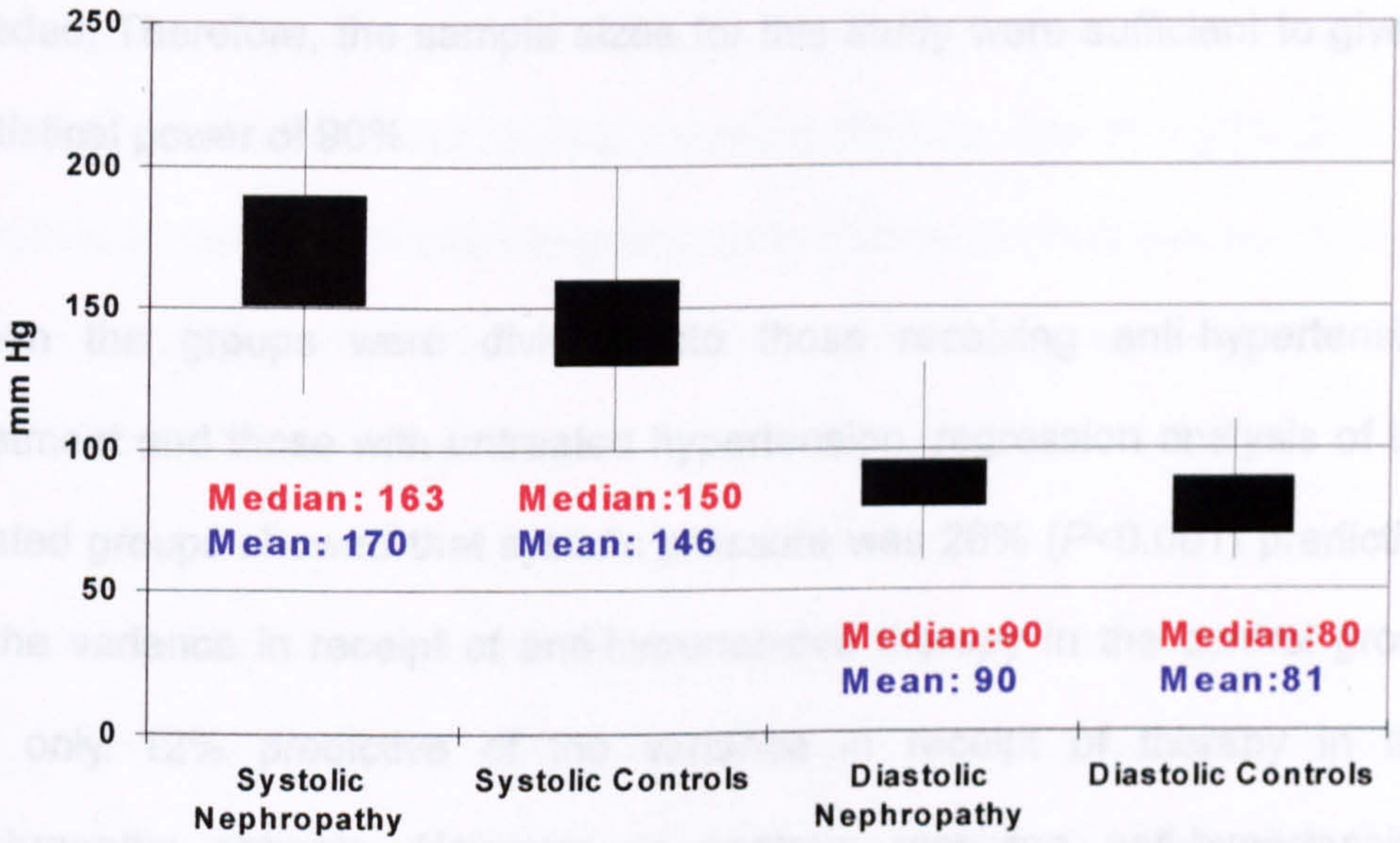


Figure 7.5: Blood pressure of Type 2 nephropathy patients and controls

Regression analysis demonstrated that in nephropathy patients systolic pressure was 26% ($P<0.001$) predictive of variance in hypertension but in the control group it was 49% predictive of the variance in hypertension ($P<0.001$). Diastolic pressure was only 13% ($P<0.05$) predictive of the variance in hypertension in controls but not predictive in the nephropathy group ($R^2 = 0.009$; P= N.S.). The higher predictive value of systolic pressure in the control group indicates that although this group had a lower mean systolic pressure than the nephropathy patients, that systolic hypertension in

control patients is more predominant than diastolic hypertension. However, in the nephropathy group, patients had both systolic and diastolic hypertension.

The proportion of patients with hypertension in each group was used to calculate the power of the study using the method described by Lowe, 1993. At 90% statistical power with $P \leq 0.05$, 40 patients in each group would be needed. Therefore, the sample sizes for this study were sufficient to give a statistical power of 90%.

When the groups were divided into those receiving anti-hypertensive treatment and those with untreated hypertension, regression analysis of the treated groups showed that systolic pressure was 26% ($P<0.001$) predictive of the variance in receipt of anti-hypertensive therapy in the control group but only 12% predictive of the variance in receipt of therapy in the nephropathy patients. However, in controls receiving anti-hypertension treatment diastolic pressure was 11% ($P<0.05$) predictive of the variance in treatment in comparison to 1% (P=N.S.) in the nephropathy group. Multiple regression analysis showed that diastolic and systolic pressures together were 26% predictive of anti-hypertensive therapy in treated control patients with only systolic pressure being significant ($P<0.001$). These results can be interpreted as showing that in both groups systolic pressure was more indicative of the lack of anti-hypertensive treatment than diastolic pressure.

Body mass index (BMI) was 22% (P<0.005) predictive of the variance in treatment of hypertension in the nephropathy group but only 4% (P=N.S.) in the control group. Multiple regression analysis showed that systolic pressure and BMI together were 36% (systolic pressure: P<0.01, BMI: P<0.05) predictive in the nephropathy group but only 26% (systolic pressure: P<0.05, BMI P=N.S.) predictive in controls. Multiple regression analysis using defaulting from clinic visits, presence of retinopathy, smoking history, systolic pressure and BMI as independent variables showed a combined predictive value of 39% with only the systolic pressure (P=0.01) and BMI (P<0.005) as significant predictors of anti-hypertensive treatment in nephropathy patients. In the control group these factors combined to give a predictive value of 27% with only systolic pressure being statistically significant (P<0.05).

In patients with untreated hypertension there were no similarities between the control and nephropathy groups. In nephropathy patients systolic pressure was 15% (P<0.05) predictive and BMI was 22% (P<0.001) predictive of the variance in lack of anti-hypertensive therapy unlike the control group where neither of these parameters were significant. Multiple regression analysis demonstrated that systolic pressure and BMI together were 31% (systolic pressure; P=N.S., BMI: P<0.05) predictive of untreated hypertension in the nephropathy group. Multiple regression analysis using defaulting from clinic visits, presence of retinopathy, smoking history, systolic pressure and BMI as independent variables showed that none of these variables were statistically significant predictors in controls but when combined were 42% predictive of lack of anti-hypertensive treatment.

However, in the nephropathy group these same factors were only 34% predictive of untreated hypertension with BMI (P<0.05) being the only statistically significant factor.

7.5.4. Other Contributory Factors

A higher percentage of the nephropathy group (47%; n=22) had defaulted from routine diabetic clinic follow up compared to 14% (n=6) of controls (P<0.001). There was no significant statistical difference in current smokers between the groups. However, more nephropathy patients (n=24; 52%) had a history of smoking in comparison to the controls (n=11; 25%) (P<0.01). Defaulting from clinic visits increased the risk of developing nephropathy (odds ratio = 5.8) as did a history of smoking (odds ratio = 3.3).

There was a weak positive correlation (r=0.37) between age at referral for nephrological assessment and serum creatinine levels in the nephropathy group (P<0.05) but not in control patients (Figure 7.6).

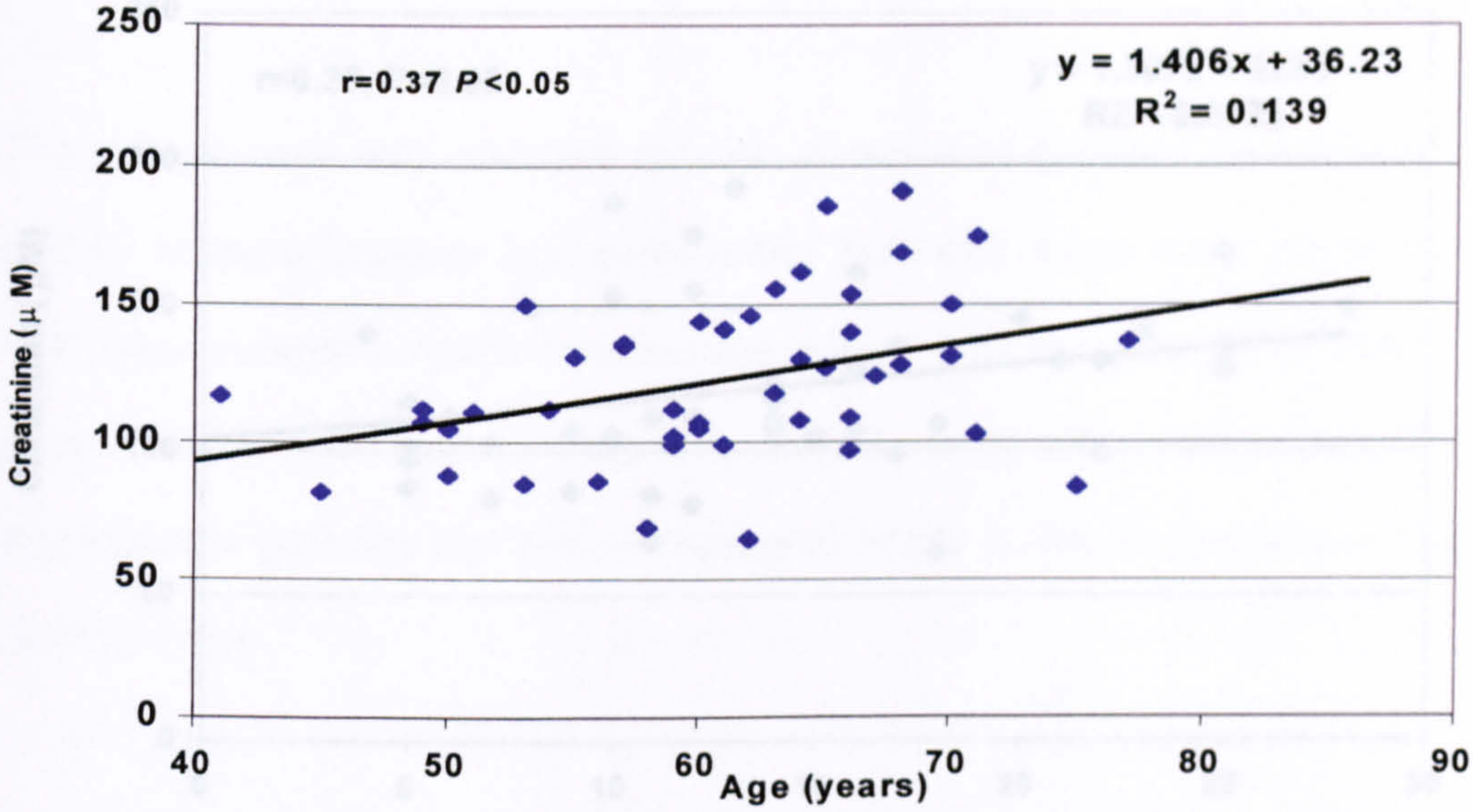


Figure 7.6: Relationship between age and serum creatinine in Type 2 nephropathy patients

Linear regression demonstrated that age at nephrological assessment predicted 14% towards the variance in serum creatinine concentrations.

Similarly, there was a weak positive correlation (r=0.29) between duration of diabetes and serum creatinine in nephropathy patients (*P*=0.05) but no correlation between these parameters in the controls (r=0.08, *P*=N.S.) (Figure 7.7). Linear regression analysis showed that duration of diabetes was only 8% predictive of the variance in creatinine levels in the nephropathy patients.

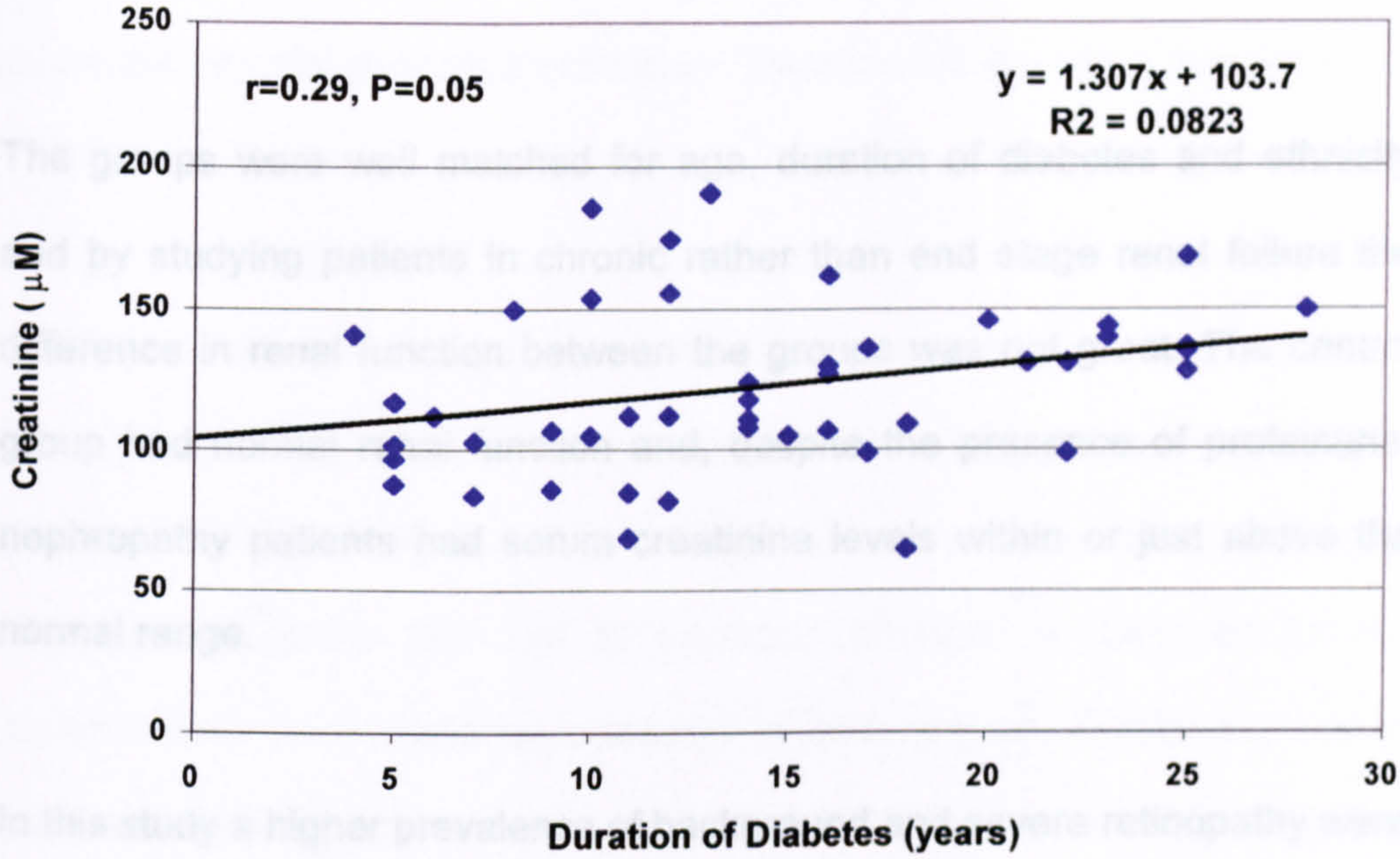


Figure 7.7: Relationship between duration of diabetes and serum creatinine in Type 2 nephropathy patients

There was no correlation between either creatinine and systolic pressure or creatinine and HbA1 at referral in either group.

7.6 Discussion

The groups were well matched for age, duration of diabetes and ethnicity and by studying patients in chronic rather than end stage renal failure the difference in renal function between the groups was not great. The control group had normal renal function and, despite the presence of proteinuria, nephropathy patients had serum creatinine levels within or just above the normal range.

In this study a higher prevalence of background and severe retinopathy were found in the nephropathy group than in controls. This was similar to a recent study in Japanese Type 2 patients which showed that those with macroproteinuria have a higher prevalence of background and proliferative retinopathy than patients who are normoalbuminuric or microalbuminuric (Tanaka *et al*, 1998).

The only differences in macrovascular complications were the high prevalence of hypertension, the low prevalence of anti-hypertensive treatment and the higher levels of mean systolic and diastolic pressures in nephropathy patients in comparison to controls. These findings are similar to a study in Korean Type 2 patients which demonstrated a higher prevalence of hypertension and higher systolic and diastolic blood pressures in patients progressing to microalbuminuria than those who remained normoalbuminuric (Park et *al*, 1998). The lack of anti-hypertensive therapy in the nephropathy group was disturbing as control of hypertension is recognised as the most

effective way of slowing the decline in renal function in patients with microalbuminuria and overt proteinuria (Kasiske *et al*, 1993; Ahmad *et al*, 1997).

The comparison of treated and untreated hypertensive patients demonstrated that not only was systolic pressure a major factor in predicting treatment of hypertension in both groups but also a lack of treatment in the nephropathy group (but not in controls) presumably because of the predominance of systolic hypertension in both groups. Diastolic pressure was only predictive in controls with treated hypertension which may be secondary to the lower mean diastolic pressure in this group (i.e.: as there were fewer patients with diastolic pressures above the upper limit of normal, those that were represented a significant minority in terms of uncontrolled hypertension). These results suggest that in control subjects with treated hypertension, the level of blood pressure control was poor. While in the nephropathy group, control of systolic pressure is not as effective as control of diastolic pressure. However, the comparison of results for untreated hypertensive patients must be regarded with some caution as there were only three controls in this subgroup and larger numbers could alter the results and interpretation.

Body mass index also played a significant role in predicting anti-hypertensive treatment and the lack of this treatment in nephropathy patients but not in the control group. This may reflect the significantly larger BMI in the nephropathy group with treated hypertension. The apparently combined

enhancing effects of systolic pressure and BMI on both treatment of hypertension and lack of the same may indicate two sides of the same problem i.e.: patients who are overweight are more likely to be hypertensive and the larger the patient the more difficult it is to control blood pressure.

A previous study in Wolverhampton has demonstrated that defaulting from routine diabetic clinic visits increases the chance of patients developing diabetic complications (Hammersley *et al*, 1985). Therefore, the results of this study showing that control subjects were less likely to default and have fewer microvascular complications in comparison to people with nephropathy was not surprising. The increased risk of developing nephropathy associated with defaulting from clinic visits confirms the previous findings.

The difference between the two groups in the prevalence of current smokers and those with a history of smoking, and the increased risk that a history of smoking confers on the development of nephropathy are similar to the results of a study by Bruno and colleagues in 1996, who demonstrated that in Type 2 diabetic patients a history of smoking increased the risk of developing nephropathy in comparison to current smokers. This apparent anomaly is possibly due to ex-smokers having smoked for a longer period of time than current smokers.

The correlation between age at referral and serum creatinine, and duration of diabetes and serum creatinine in the nephropathy group but not in control

subjects was particularly interesting as the groups were well matched for both age and duration of diabetes. There may be four factors influencing these results. The nephropathy patients may be more prone to vascular disease than the control group of the same age, and their renal disease and higher blood pressure levels may be reflective of this. The fact that patients with nephropathy were more likely to have smoked may have put them at a higher risk of developing vascular disease. Type 2 patients who develop nephropathy at the same age and duration of diabetes as matched controls may be more susceptible to this complication due to genetic factors influencing renal function or blood pressure control, or both. The link between age and serum creatinine levels may be due to the natural effect of age in reducing effective renal plasma flow and subsequent reduction in renal function. This effect has been observed to be greater in newly diagnosed Type 2 patients than in non-diabetic people (Ritz and Stephanski, 1996).

The real duration of diabetes in Type 2 patients is very difficult to ascertain. In this study the exclusion of patients presenting with microvascular complications within the first two years of diagnosis has decreased the confounding influence of undiagnosed diabetes. None the less, duration of diabetes is essentially an undefinable variable in Type 2 patients. However, bearing this in mind, duration of diabetes has been shown previously to be related to macroalbuminuria in Type 2 patients (Bruno *et al*, 1996).

The relatively small numbers of patients in this study make it difficult to access the true extent of the association between certain factors and nephropathy and this was one of the limiting factors to the research. Larger patient numbers would have undoubtedly identified factors which have a lesser effect but may still be very relevant.

The possible bias in selection of control subjects previously referred to in relation to Type 1 control subjects is also relevant to Type 2 control patients i.e.: only patients attending for annual review examination were used to select control subjects therefore potentially excluding patients who had defaulted from clinic and the collection of data on control subjects occurred five years after the start of collecting data on the nephropathy group during which time the clinical approach to hypertension treatment and glycaemic control may have changed.

7.7 Conclusions

1 Type 2 nephropathy patients had a higher prevalence of retinopathy, including severe retinopathy, than controls.

2 Type 2 nephropathy patients had a higher prevalence of hypertension, higher systolic and diastolic pressures and less anti-hypertensive treatment than controls. Therefore, accept H_1 (1): Nephropathy patients have more diabetic complications and a higher prevalence of hypertension than matched controls.

3 Duration of diabetes and increasing age are significant factors associated with the development of nephropathy in susceptible patients.

4 The presence of hypertension was a risk factor for the development of nephropathy.

5 A history of smoking was a risk factor for the development of nephropathy in Type 2 patients.

6 Defaulting from routine diabetic clinic follow up was a risk factor for developing nephropathy in Type 2 patients. Therefore, accept H_0 (2): There are factors other than glycaemic control, the presence of hypertension and duration of diabetes that put Type 2 patients at risk

of developing nephropathy that can be identified during routine clinical practice.

7 Increasing body mass index and untreated hypertension were associated with the presence of nephropathy.

Chapter 8

A Comparison of Diabetic Nephropathy in Type 2 Patients from Different Ethnic Groups

8.1 Introduction

The 1991 census identified that 3% of the British population were of Indo-Asian origin (Cruickshank, 1993). However, in Wolverhampton the same census has shown that 13% of our population are Indo-Asian and 5% Black [Indo-Asian and Black refer to the definitions used in this thesis not those of the OPCS (see Section 7.4)] (Office of Population Censuses and Survey, 1991). In 1987, a survey of 549 diabetic clinics in the U.K. estimated that 4.3% of patients attending were of Indo-Asian origin and 2.1% were Black (Goodwin *et al*, 1987). From data collected to find control subjects for the earlier chapters, 139 (15%) Indo-Asian patients and 50 (6%) Black patients attended the diabetic clinic in Wolverhampton for annual review examination out of 908 patients over a four year period, however, this represents less than half of the diabetic clinic population.

The Southall and Coventry studies have demonstrated that Indo-Asian people have a four times increased risk of developing diabetes than the indigenous White population of the U.K. (Mather and Keen, 1985; Simmons *et al*, 1989). Both the Coventry study and the UK Prospective Diabetes Study have shown that Indo-Asian patients develop Type 2 diabetes at a younger age than White patients (Simmons and Powell, 1993; UK Prospective Diabetes Study Group, 1994). In 1988, Allawi and co-workers found that Indian diabetic patients living in the U.K. had a higher prevalence of microalbuminuria than people of

European origin (Allawi *et al*, 1988). It has also been shown that Indo-Asian men with diabetes were twice as likely to develop chronic renal failure than White diabetic men and that the incidence of end-stage renal failure secondary to diabetes in Indo-Asian people is ten times that of White diabetic patients (Burden *et al*, 1992; Gujral *et al*, 1997).

A previous study in Wolverhampton has shown that people of Black origin had a higher incidence of diabetes than White people living in other parts of the U.K. (Odugbesan, *et al*, 1989). In the U.S.A., Black patients with Type 2 diabetes are known to have a 2.6 higher incidence of end stage renal disease than White patients (Cowie, *et al*, 1989). Perneger and colleagues (1994) found that the odds for Black patients developing end stage renal disease was 7.4 times greater than in White patients. In one renal unit in the U.K., 45% of Type 2 patients receiving dialysis for end stage renal failure due to nephropathy were either Indo-Asian or Black (Grenfell *et al*, 1988).

Whether there are specific risk factors, predictors of, or indeed differences in the development of nephropathy in Indo-Asian, Black or White diabetic patients is unclear. This study is designed to ascertain whether there are any differences between these three ethnic groups which may relate to the development of nephropathy.

8.2 Objectives

1 To determine whether there were differences in the presentation of nephropathy between White, Indo-Asian and Black patients.

2 To determine whether there were differences in the prevalence of diabetic complications between the groups.

3 To determine whether there were risk factors for nephropathy specific to any of the ethnic groups.

4 To determine whether there were factors associated with nephropathy specific to any of the ethnic groups.

8.3 Hypotheses

1 H_o: There were no differences in the presentation of nephropathy between White, Indo-Asian and Black Type 2 diabetic patients.

H_1: There were differences in the presentation of nephropathy between White, Indo-Asian and Black Type 2 diabetic patients.

2 H_o: There were no differences in the prevalence of diabetic complications between White, Indo-Asian and Black Type 2 diabetic patients.

H_1: There were differences in the prevalence of diabetic complications between White, Indo-Asian and Black Type 2 diabetic patients.

3 H_o: There were no risk factors for nephropathy specific to either White, Indo-Asian or Black Type 2 diabetic patients.

H_1: There were risk factors for nephropathy specific to either White, Indo-Asian or Black Type 2 diabetic patients.

4 H_o: There were no factors associated with nephropathy specific to either White, Indo-Asian or Black diabetic Type 2 patients.

H_1: There were factors associated with nephropathy specific to either White, Indo-Asian or Black diabetic Type 2 patients.

8.4 Methods

From the index population of patients referred for nephrological assessment from the diabetic clinic, Type 2 patients with nephropathy were divided into three groups dependent on ethnic origin; White (defined as people with genetic origins in Europe, irrespective of place of birth), Indo-Asian (defined as people with genetic origins from the Indian subcontinent, irrespective of place of birth) and Black (defined as people with genetic origins from Africa irrespective of place of birth) and matched according to age, gender, duration of diabetes and diabetes treatment. For the initial analysis patients on renal replacement therapy or with serum creatinine levels greater than 300 μmol l^{-1} were omitted from the analysis to remove bias due to advanced disease. A further analysis was performed to validate the results of Chi^2 tests by using larger numbers of patients; this included patients with serum creatinine levels above 300 μmol l^{-1} and those on RRT.

Data were collected as in Chapter 4 for both groups of patients i.e.: information on diabetes history, treatment and complications, renal disease, hypertension and anti-hypertensive treatment; hypertension was defined as systolic pressure >140 mm Hg and / or diastolic pressure >90 mm Hg. Nephropathy was diagnosed on clinical grounds i.e.: they had to have either proteinuria on "Albustix" testing plus diabetic retinopathy or confirmation of diagnosis by renal biopsy. Diabetic control was determined using glycated haemoglobin (HbA1)

results at either nephrological assessment or annual review examination (controls). HbA1 results over a four-year period were collected and comparisons were made between the groups. A point prevalence i.e.: prevalence at a single examination (nephrological assessment) was determined for diabetes complications and other factors that may be predictive of the development of nephropathy.

Duration of diabetes to the onset of proteinuria, retinopathy and hypertension were also determined and compared between the groups to assess whether there were any differences in development of these complications.

A comparison was made between patients with treated and untreated hypertension to determine whether there were factors which contributed towards lack of anti-hypertensive therapy which were specific to any of the ethnic groups.

Descriptive statistics (means $\pm$standard deviations), Chi^2 tests, analysis of variance between and within the groups, Pearson correlation and linear and multiple regression analysis were performed using SPSS statistics package. The chosen level of significance was $P \leq 0.05$ for one and two-tailed tests.

8.5 Results

From the original cohort of 102 Type 2 patients with nephropathy 61 patients were included in the comparison of ethnic groups: 17 Indo-Asian, 10 Black and 34 White. The groups were well matched for age, sex, duration of diabetes and diabetes treatment. Indo-Asian patients had the shortest duration of diabetes at referral: mean 11 (±5) years with a median of 10 years (*P*=N.S.). (Table 8.1). All but one of the Black patient were on insulin treatment, unlike the other two groups where up to 35% were on oral therapy and none were on diet treatment only.

	INDO-ASIAN n (%)	BLACK n (%)	WHITE n (%)	*P*
NUMBER	17	10	34	
MALE	10 (59)	7 (70)	23 (68)	
FEMALE	7 (41)	3 (30)	11 (32)	N.A.
AGE (YEARS)				
RANGE	41-68	49-71	45-83	
MEAN (±SD)	59 (7)	60 (8)	64 (8)	N.S.
MEDIAN	60	59	64	
DURATION DM (YEARS)				
RANGE	3-20	7-25	1-28	
MEAN (±SD)	11 (5)	16 (5)	14 (7)	N.S.
MEDIAN	10	16	15	
DIABETES TREATMENT				
DIET	0	0	0	
ORAL	6 (35)	1 (10)	9 (26)	N.A.
INSULIN	11 (65)	9 (90)	25 (74)	

D.M.: Diabetes mellitus S.D.: Standard deviation N.S.: Not significant
N.A.: Not applicable (Expected frequency $\chi^2 < 5$) Level of significance: $P \leq 0.05$

Table 8.1: Demographics of Type 2 nephropathy patients from different ethnic backgrounds.

There was little difference in glycaemic control (as measured by HbA1 at referral) between the groups [mean (±SD) Indo-Asian: 12.6 (±4.2) %; Black: 12.7 (±2.3) %; White: 11.1 (±2.2) % (*P*=N.S.)]. Analysis of variance showed that there was no difference either within or between the groups in glycaemic control over four years (*P*=N.S.) (Figure 8.1).

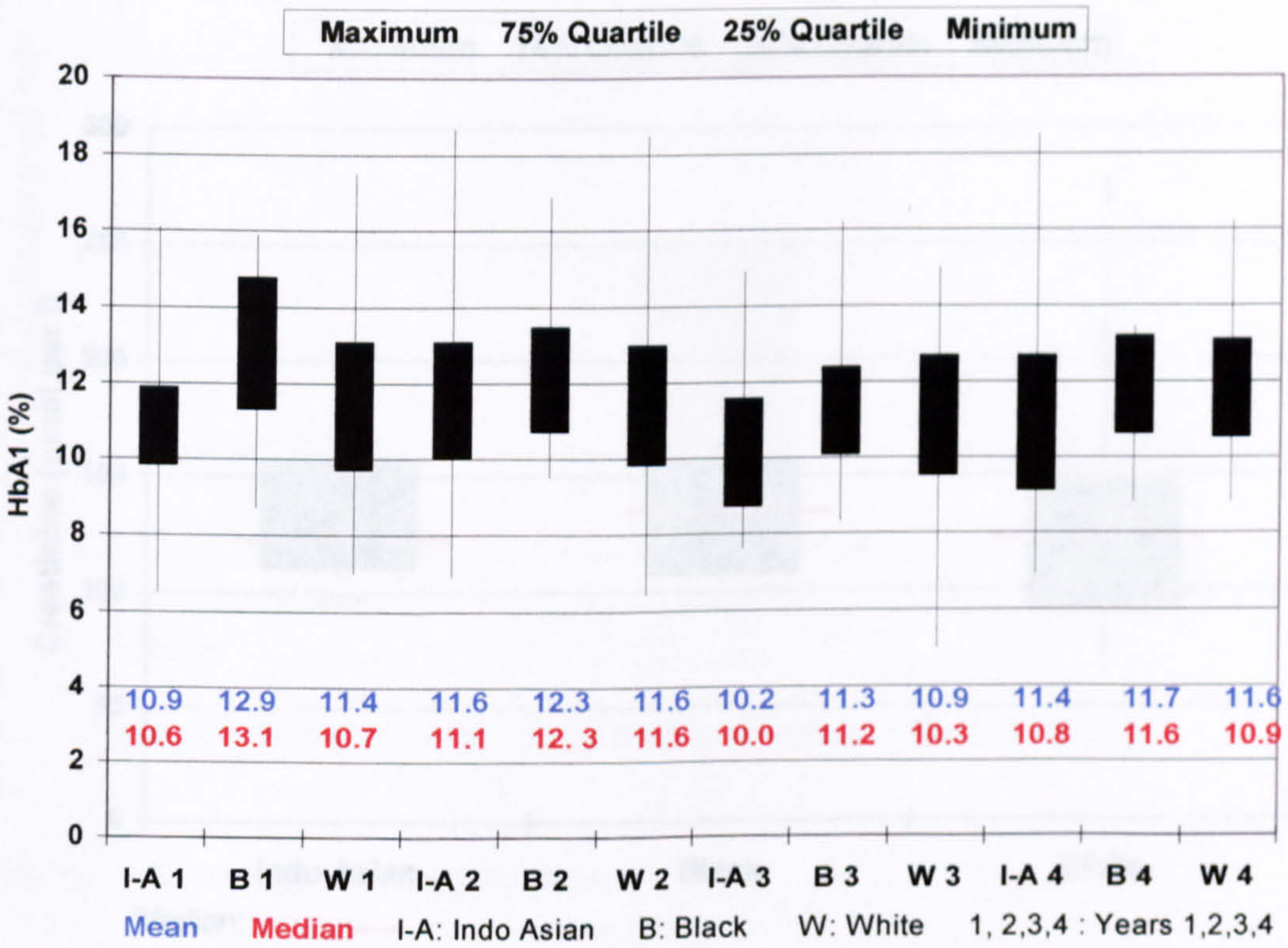


Figure 8.1: Glycaemic control over four years in Type 2 nephropathy patients of different ethnic origin

8.5.1 *Renal Function*

Mean serum creatinine levels were similar in all three groups [Indo-Asian: 131 (±30) μmol l^{-1}, Black: 135 (±30) μmol l^{-1}, White: 130 (±50) μmol l^{-1}; (*P*=N.S.)] although the range in the White group was wider than in the other two groups (Figure 8.2).

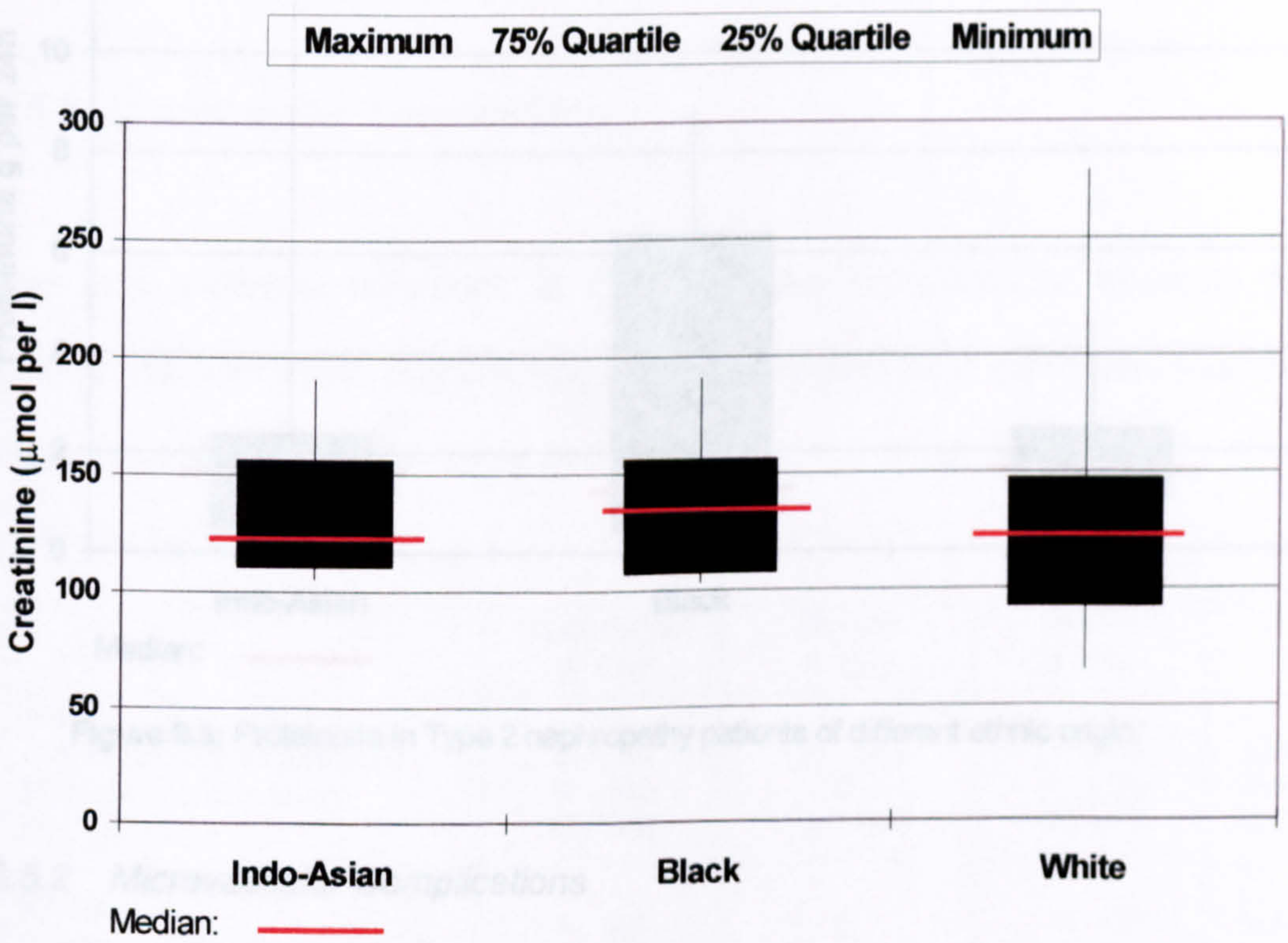


Figure 8.2: Serum creatinine concentrations in Type 2 nephropathy patients from different ethnic groups

There was no statistical difference in mean proteinuria between the groups (Indo-Asian: 2.3 (±3.1) g $24h^{-1}$, Black: 2.9 (±3.5) g $24h^{-1}$, White: 1.8 (±1.1) g

$24h^{-1}$) but ranges varied and the inter-quartile range in the Black group was wider than the other two groups (Figure 8.3).

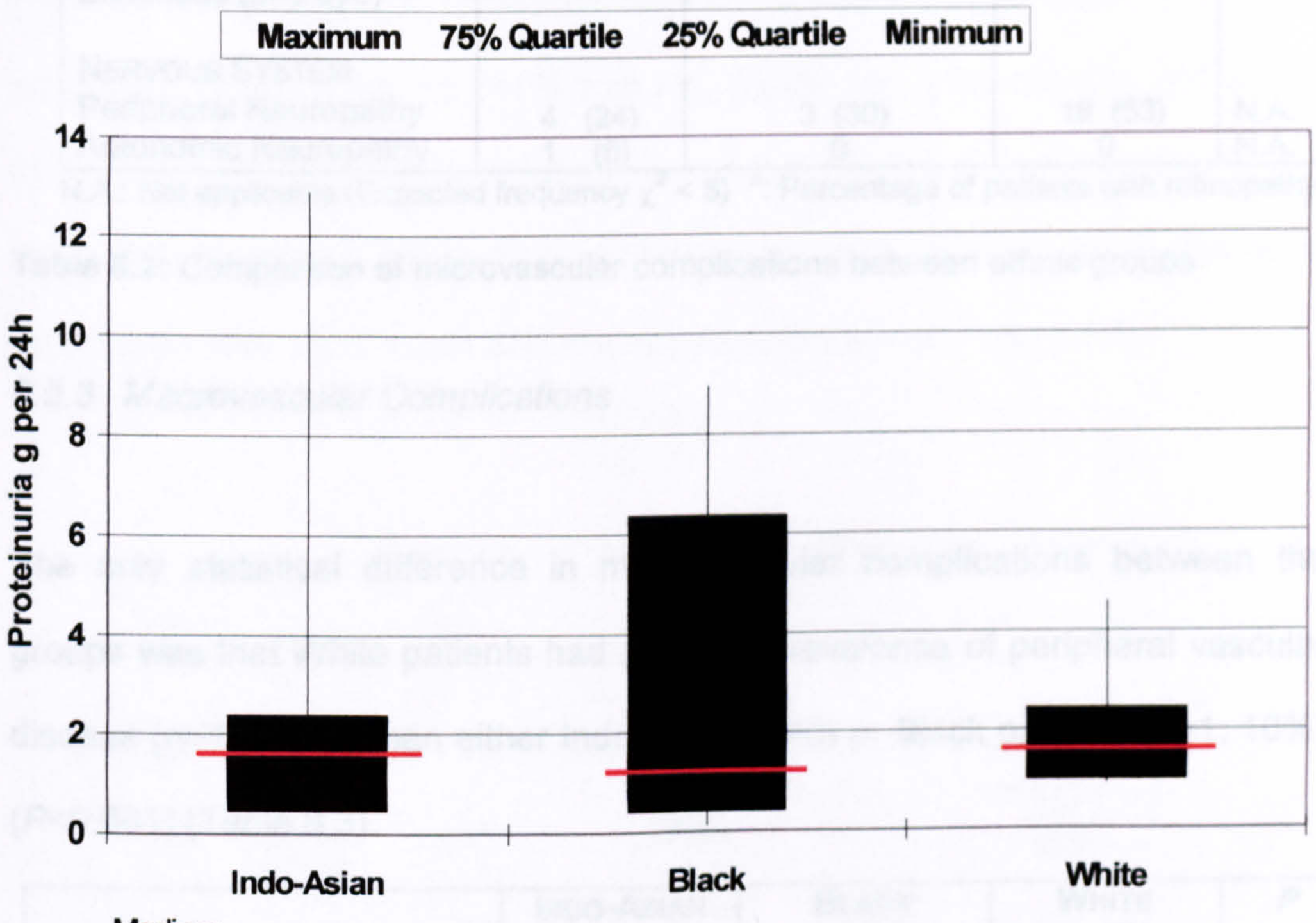


Figure 8.3: Proteinuria in Type 2 nephropathy patients of different ethnic origin

8.5.2 Microvascular Complications

Microvascular complications were similar in all three groups (Table 8.2).

	INDO-ASIAN n (%)	BLACK n (%)	WHITE n (%)	*P*
EYES				
Retinopathy	15 (88)	9 (90)	34 (100)	N.A.
Severe Retinopathy*	9 (60)	4 (44)	19 (56)	N.A.
Laser Photocoagulation*	6 (40)	4 (44)	17 (50)	N.A.
Blindness (one eye)	1 (6)	0	0	N.A.
NERVOUS SYSTEM				
Peripheral Neuropathy	4 (24)	3 (30)	18 (53)	N.A.
Autonomic Neuropathy	1 (6)	0	0	N.A.

N.A.: Not applicable (Expected frequency $\chi^2 < 5$) *: Percentage of patients with retinopathy

Table 8.2: Comparison of microvascular complications between ethnic groups

8.5.3 Macrovascular Complications

The only statistical difference in macrovascular complications between the groups was that White patients had a higher prevalence of peripheral vascular disease (n=18; 53%) than either Indo-Asian (n=0) or Black patients (n=1; 10%) (*P*<0.001) (Table 8.3).

	INDO-ASIAN n (%)	BLACK n (%)	WHITE n (%)	*P*
HEART				
Ischaemic Heart Disease	3 (18)	2 (20)	9 (26)	N.A.
Myocardial Infarction	1 (6)	0	5 (15)	N.A.
Hypertension	17 (100)	10 (100)	33 (97)	N.S.
BRAIN				
Transient Ischaemic Attack	1 (6)	0	1 (3)	N.A.
Cerebrovascular Accident	0	2 (20)	3 (9)	N.A.
PERIPHERIES				
Peripheral Vascular Disease	0	1 (10)	18 (53)	N.A.
Ulcers (foot/leg)	1 (6)	1 (10)	9 (27)	N.A

N.S.: Not significant N.A.: Not applicable (Expected frequency $\chi^2 < 5$)
Level of significance: *P* <0.05

Table 8.3: Comparison of macrovascular complications between ethnic groups

Blood pressure levels were similar across all three groups. Mean systolic pressure demonstrated that each group had systolic hypertension (Indo-Asian: 172 (±25) mm Hg; Black: 173 (±23) mm Hg; White: 173 (±23) mm Hg [*P*=N.S.]). Mean diastolic pressures were just above the upper limit of normal in all groups (Indo-Asian: 93 (±15) mm Hg; Black: 92 (±18) mm Hg; White: 94 (±15) mm Hg [*P*=N.S.]). In White patients the median systolic pressure was lower than the mean: 165 mm Hg in comparison to 173 mm Hg, unlike the other two groups where the mean and median were the same (Figure 8.4).

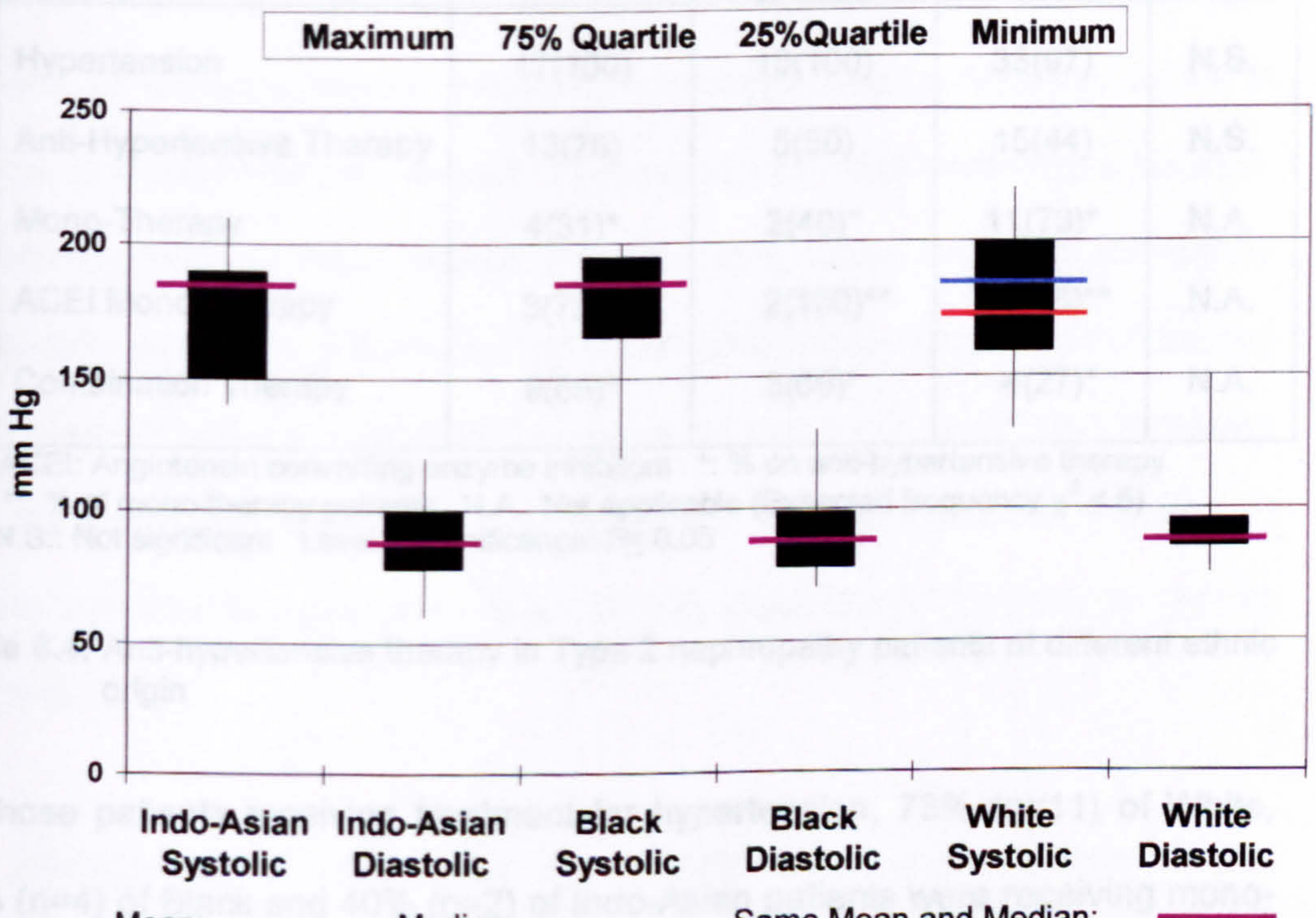


Figure 8.4: Blood pressures in nephropathy patients from different ethnic origins

The inter-quartile range for systolic pressure was smallest in the Black patients and for diastolic pressure was smallest in White patients.

All Indo-Asian and Black patients and 97% of the White group had hypertension but only 44% (n=15) of White patients were receiving anti-hypertensive medication in comparison to 65% (n=11) Indo-Asian patients and 50% (n=5) of Black patients (*P*=N.S.).

Anti-hypertensive Therapy	Indo-Asian n (%)	Black n (%)	White n (%)	*P*
Hypertension	17(100)	10(100)	33(97)	N.S.
Anti-Hypertensive Therapy	13(76)	5(50)	15(44)	N.S.
Mono-Therapy	4(31)*	2(40)*	11(73)*	N.A.
ACEI Mono-Therapy	3(75)**	2(100)**	5(46)**	N.A.
Combination Therapy	9(69)*	3(60)*	4(27)*	N.A.

ACEI: Angiotensin converting enzyme inhibitors *: % on anti-hypertensive therapy
**: % of mono-therapy patients N.A.: Not applicable (Expected frequency $\chi^2 < 5$)
N.S.: Not significant Level of significance: $P \leq 0.05$

Table 8.4: Anti-hypertensive therapy in Type 2 nephropathy patients of different ethnic origin

Of those patients receiving treatment for hypertension, 73% (n=11) of White, 31% (n=4) of Black and 40% (n=2) of Indo-Asian patients were receiving mono-therapy while the remainder were treated with a combination of different types of anti-hypertensive drugs (Table 8.4). Only 46% (n=5) of White patients on mono-therapy were receiving an ACEI in comparison to 75% (n=3) Indo-Asian

and 100% (n=2) Black patients. Indo-Asian patients had the greatest need for combination therapy (69%). All combination therapies included ACEI.

When the groups were divided into patients with treated or untreated hypertension, there were only two differences within the ethnic groups. In Indo-Asian patients the mean (±SD) systolic blood pressure was significantly higher in patients with treated hypertension (184 [±24] mm Hg) than those with untreated hypertension (163 [±11] mm Hg) (P=0.05). In White patients mean (±SD) BMI was greater in treated patients (34 [±6] kg $m^{-2)}$ in comparison to untreated patients (26 [±4] kg $m^{-2)}$ (P<0.001). There were no differences in age, duration of diabetes, serum creatinine concentrations, proteinuria, HbA1 at referral or diastolic blood pressure. Comparison of these paramaters between the ethnic groups of those treated and separately those untreated showed no statistically significant differences. However, analysis of variance of all treated and untreated patients combined demonstrated that mean (±SD) BMI in treated patients was higher (Indo-Asian: 28 (±6); Black: 30 (±2): White: 34 (±6) kg m^{-2}) than in untreated patients (Indo-Asian: 27(±2); Black: 28 (±3); White: 26 (±4) kg m^{-2} (P<0.005).

On investigation of differences in correlations between various clinical parameters, it was found that in White patients with treated hypertension there was a positive correlation between age and serum creatinine (r=0.62, P<0.05, R^2 =38%) but there were no statistically significant correlations in untreated

White patients. It should be noted that the numbers were very small in groups of treated and untreated Indo-Asian and Black patients, and therefore, correlations were not performed.

8.5.4 Other Factors

When the length of time between diagnosis of diabetes and development of retinopathy, proteinuria and hypertension were compared, Indo-Asian patients developed all three complications at an earlier duration of diabetes than either Black or White patients (Table 8.5). However, the only statistically significant difference between the groups was that Indo-Asian patients developed retinopathy at an early duration of diabetes.

Time (years)	Indo-Asian	Black	White	*P*
DM-RETINOPATHY				
Range	0*-14	2-18	0-26	
Mean (±SD)	6 (5)	11 (6)	11 (8)	<0.05
Median	6	11	13	
DM-PROTEINURIA				
Range	1-20	5-24	0-27	
Mean(±SD)	9 (6)	14 (6)	14 (8)	N.S.
Median	8	16	15	
DM-HYPERTENSION				
Range	0-16	5-24	-3-25	
Mean(±SD)	8 (5)	14 (7)	12 (8)	N.S.
Median	7	16	12	

DM: Diabetes mellitus. SD: Standard Deviation. N.S.: Not significant 0*: Onset of complication in same year as diabetes was diagnosed. A minus number: Diagnosed before diagnosis of diabetes.

Table 8.5: Time to development of complications in Type 2 nephropathy patients of different ethnic origin.

Proteinuria developed after retinopathy in all three groups and after hypertension in Indo-Asian and Black patients. In Indo-Asian patients there were positive correlations between time to development of hypertension and development of proteinuria (r=0.78, *P*<0.001) (Figure 8.5) and time to development of retinopathy and development of hypertension (r=0.85, *P*<0.001) (Figure 8.6). However, there was a non-significant correlation between onset of retinopathy and proteinuria (r=0.39, *P*=N.S.).

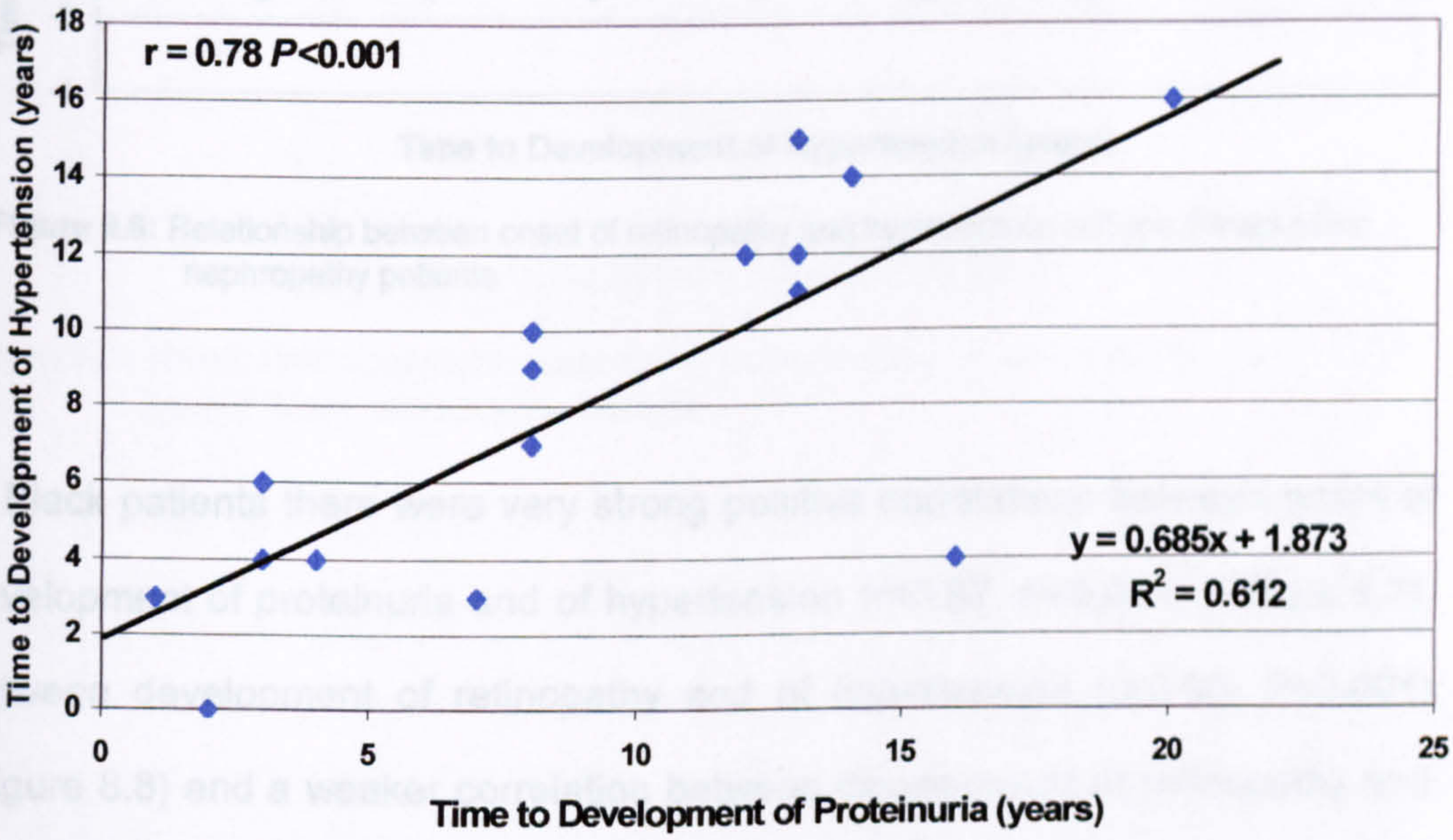


Figure 8.5: Relationship between development of proteinuria and hypertension in Type 2 Indo-Asian nephropathy patients

Regression analysis demonstrated that retinopathy was 72% predictive of the variance in time to development of hypertension in Indo-Asian patients (*P*<0.001) and hypertension was 61% predictive of the variance in time to onset of proteinuria.

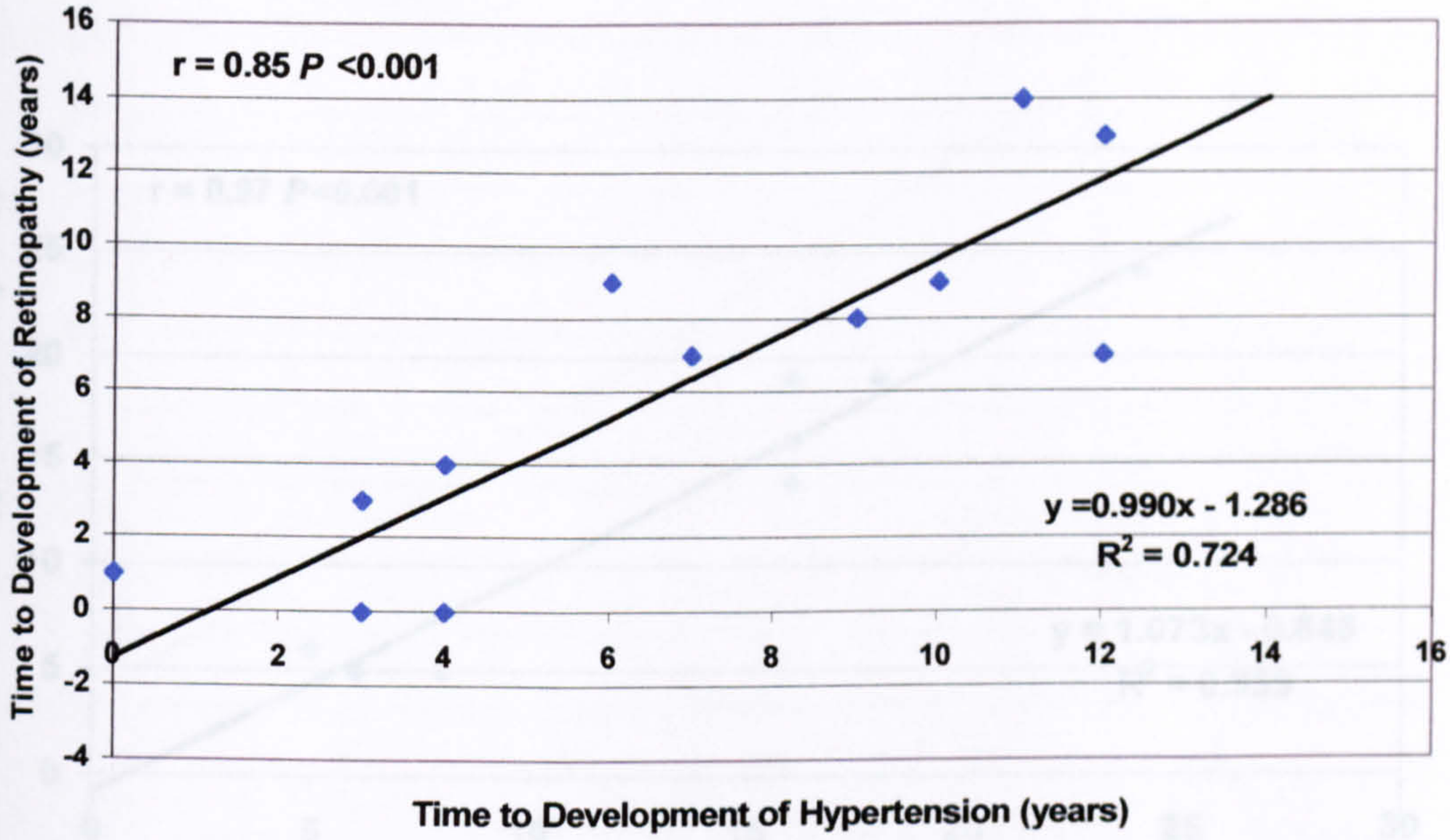


Figure 8.6: Relationship between onset of retinopathy and hypertension in Type 2 Indo-Asian nephropathy patients

In Black patients there were very strong positive correlations between onset of development of proteinuria and of hypertension (r=0.97, *P*<0.001) (Figure 8.7), between development of retinopathy and of hypertension (r=0.90, *P*=0.001) (Figure 8.8) and a weaker correlation between development of retinopathy and proteinuria (r=0.78, *P*<0.05) (Figure 8.9). Linear regression analysis demonstrated that onset of hypertension was 94% predictive of the variance in time to development of proteinuria (*P*<0.001); onset of retinopathy was 80% predictive of the variance in time to development of hypertension (*P*=0.001), and retinopathy was 61% predictive of the variance in time to onset of proteinuria (*P*<0.05).

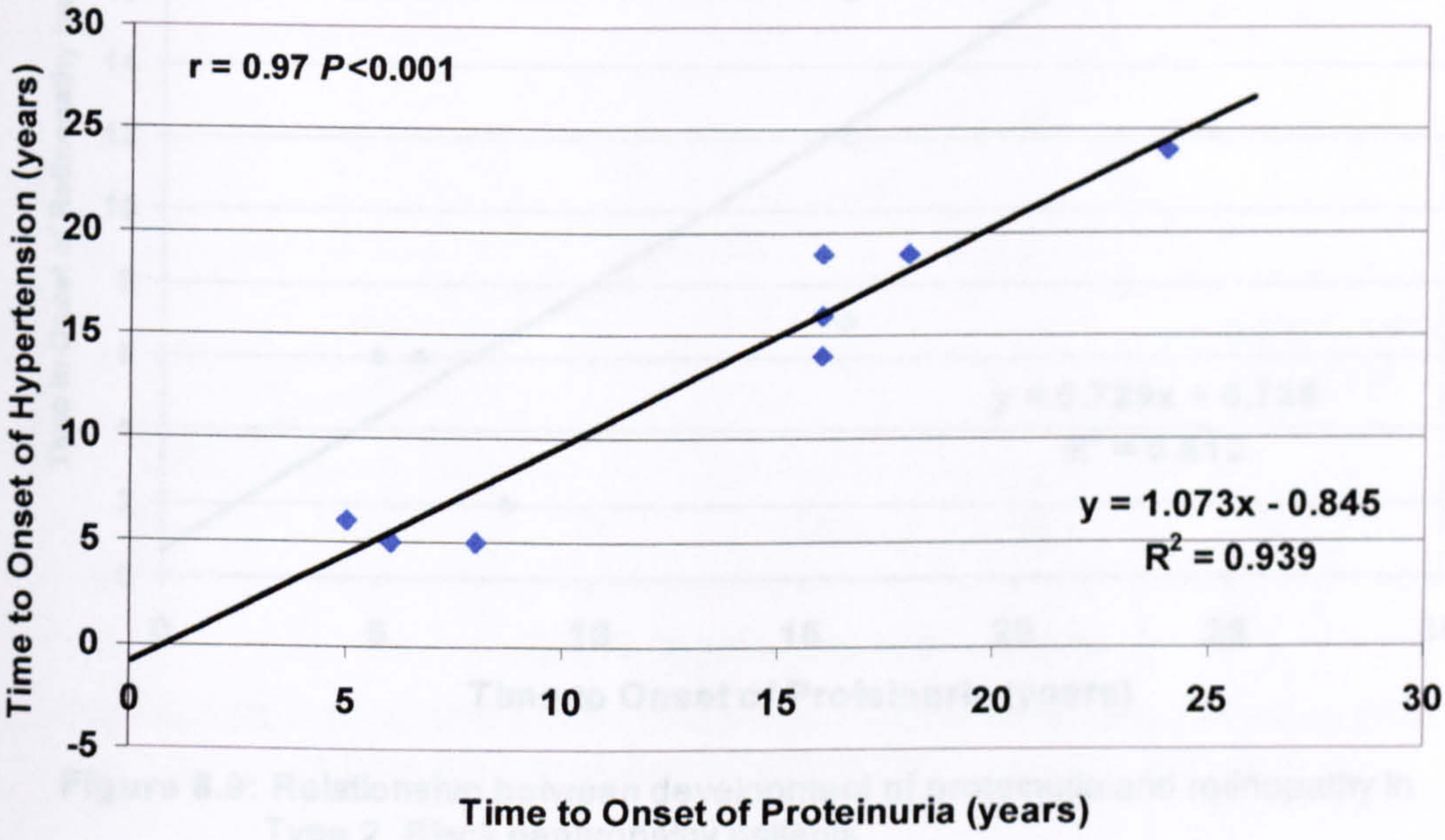


Figure 8.7: Relationship between development of hypertension and proteinuria in Type 2 Black nephropathy patients

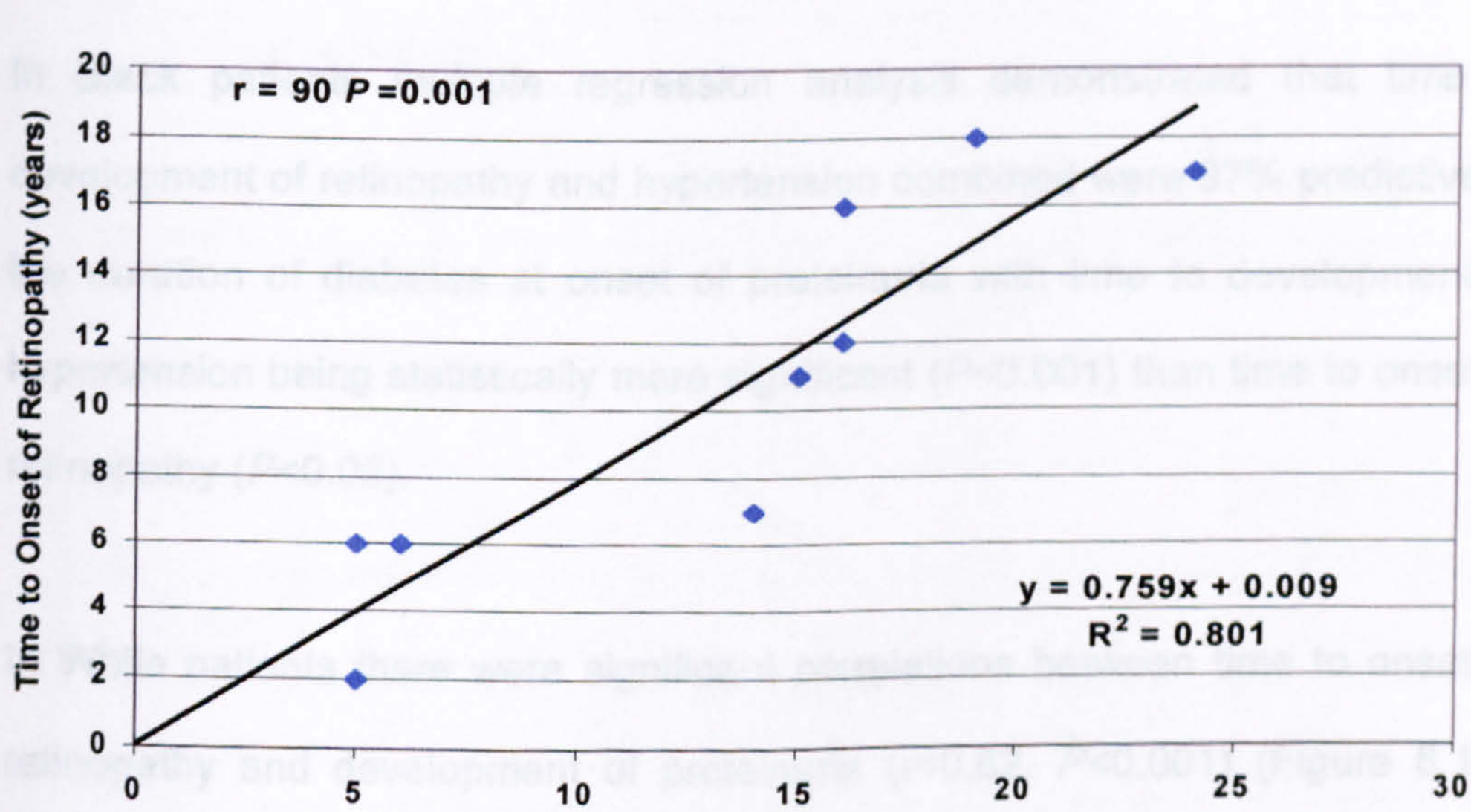


Figure 8.8: Relationship between development of retinopathy and hypertension in Type 2 Black nephropathy patients

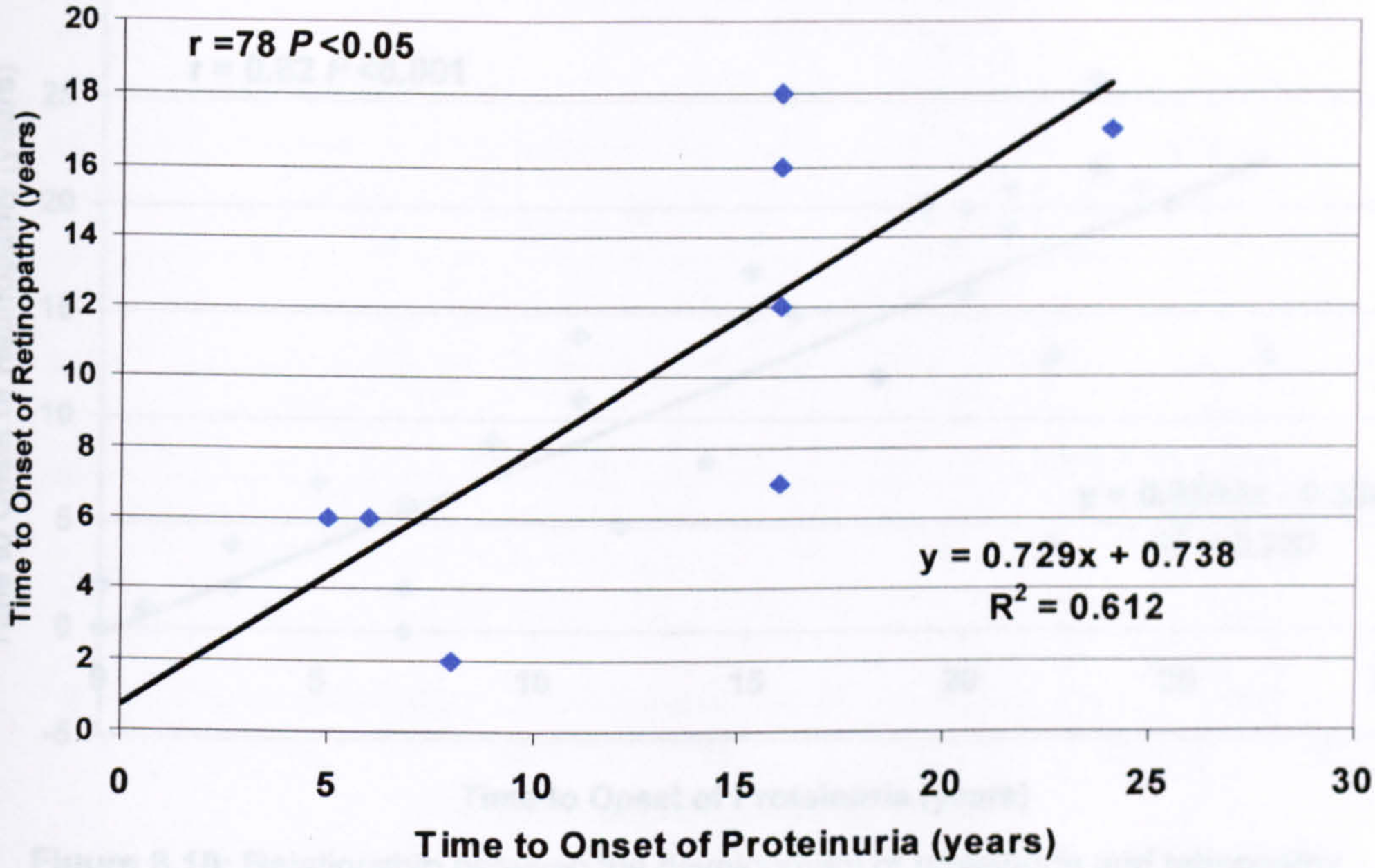


Figure 8.9: Relationship between development of proteinuria and retinopathy in Type 2 Black nephropathy patients

In Black patients multiple regression analysis demonstrated that time to development of retinopathy and hypertension combined were 97% predictive of the duration of diabetes at onset of proteinuria with time to development of hypertension being statistically more significant ($P<0.001$) than time to onset of retinopathy ($P<0.05$).

In White patients there were significant correlations between time to onset of retinopathy and development of proteinuria (r=0.82, $P<0.001$) (Figure 8.10), time to onset of hypertension and development of proteinuria (r=0.84, $P<0.001$) (Figure 8.11) and time to development of retinopathy and hypertension (r=0.83, $P<0.001$) (Figure 8.12).

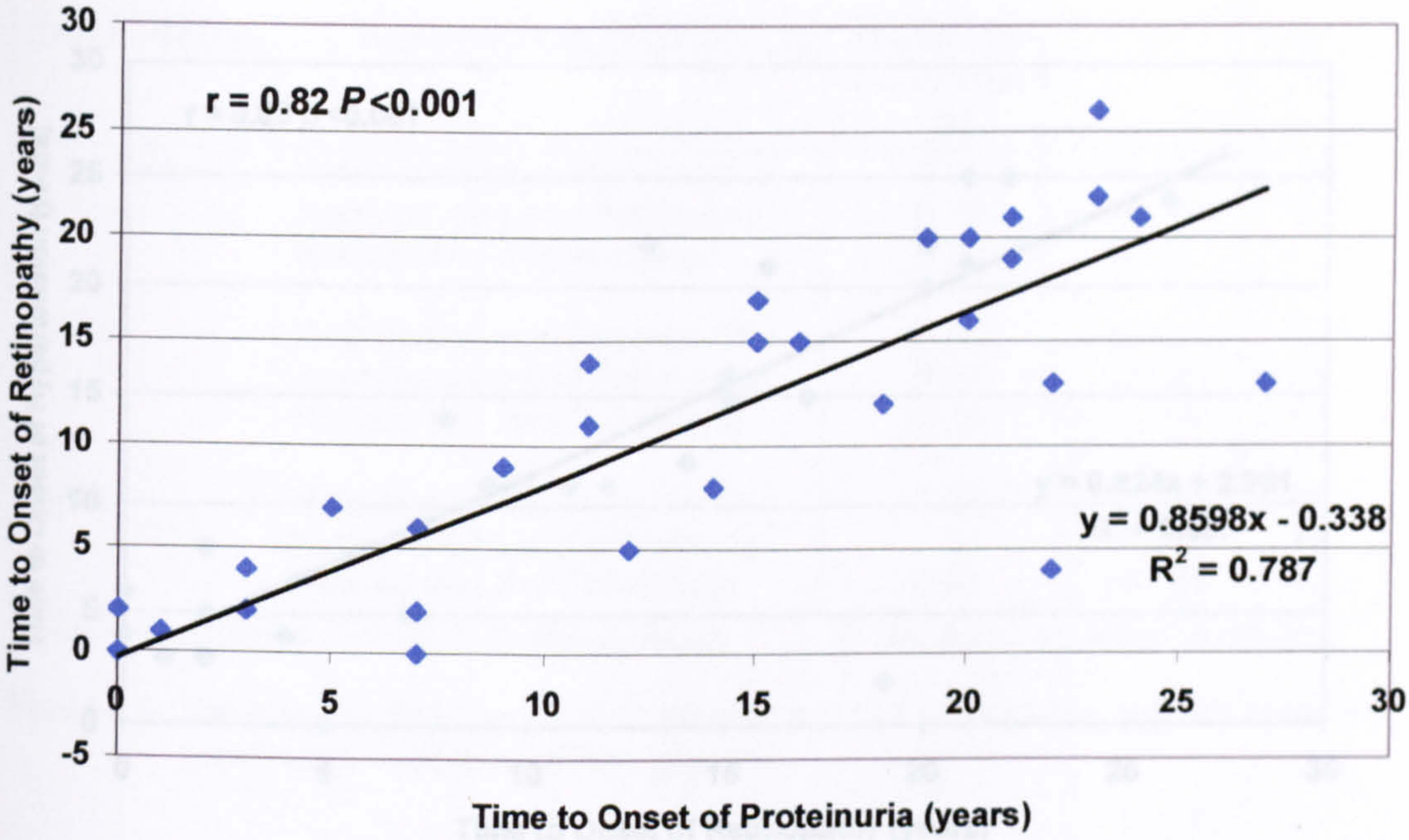


Figure 8.10: Relationship between the development of proteinuria and retinopathy in Type 2 White nephropathy patients

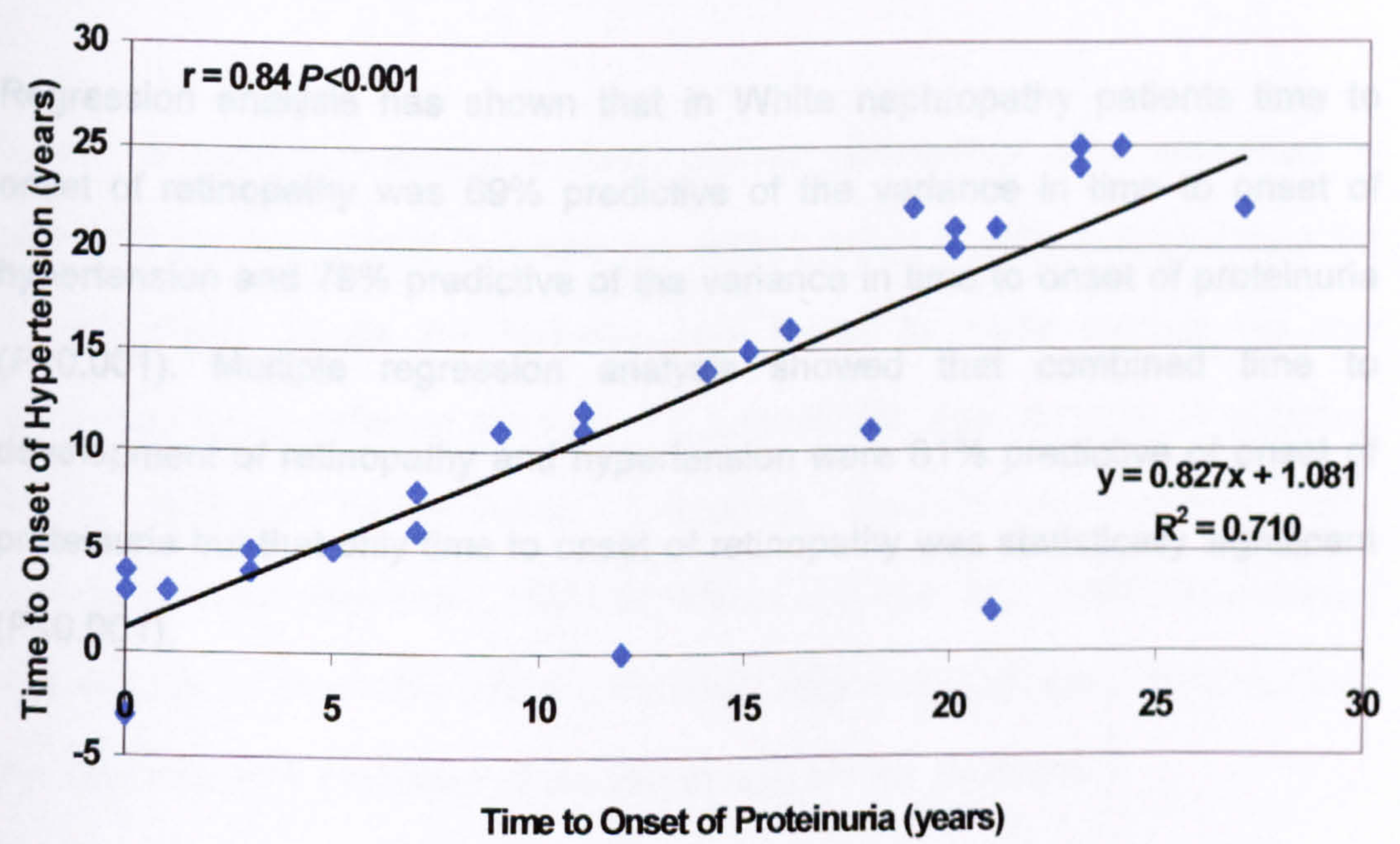


Figure 8.11: Relationship between development of proteinuria and hypertension in Type 2 White nephropathy patients

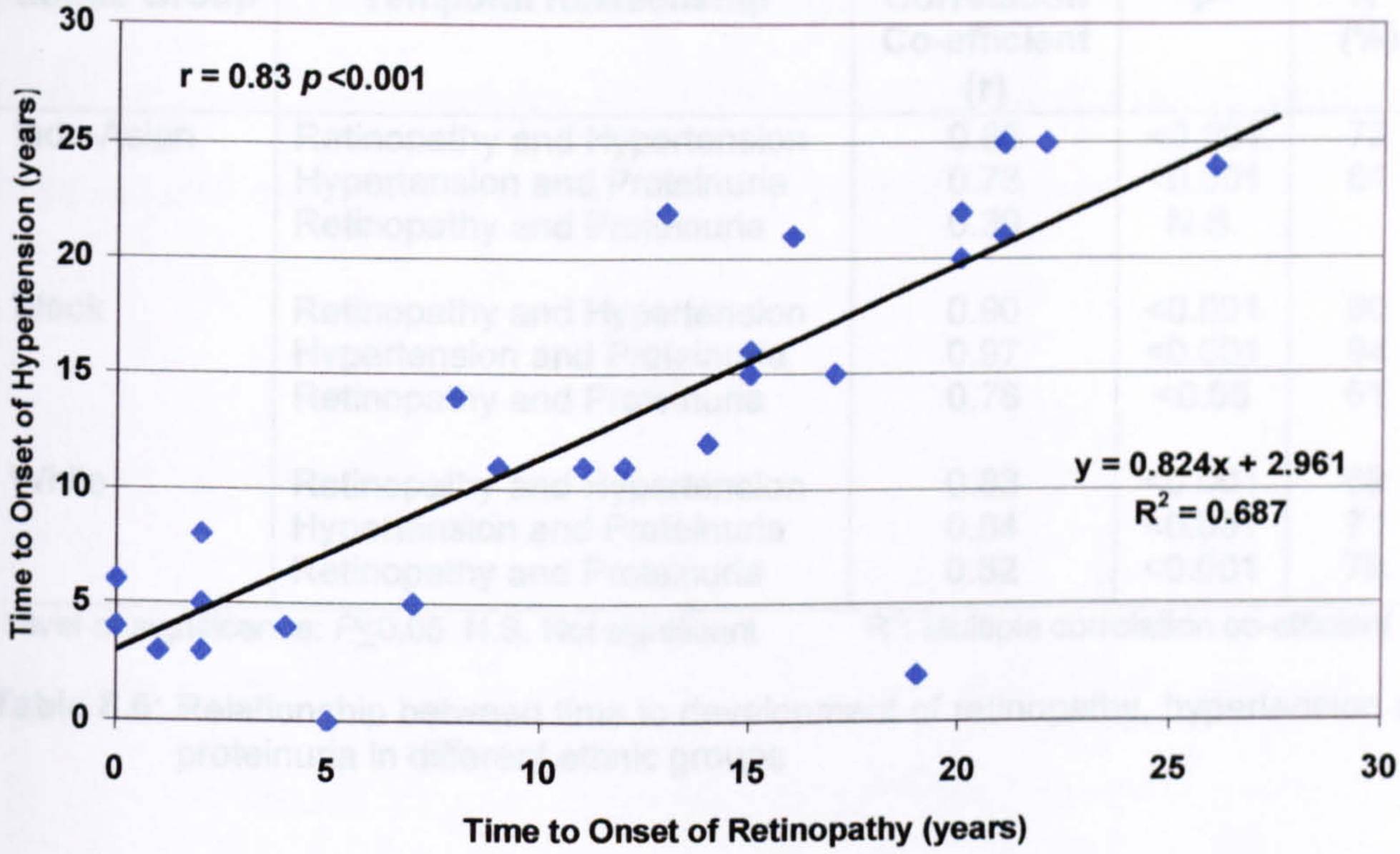


Figure 8.12: Relationship between development of retinopathy and hypertension in Type 2 White nephropathy patients

Regression analysis has shown that in White nephropathy patients time to onset of retinopathy was 69% predictive of the variance in time to onset of hypertension and 79% predictive of the variance in time to onset of proteinuria (P<0.001). Multiple regression analysis showed that combined time to development of retinopathy and hypertension were 81% predictive of onset of proteinuria but that only time to onset of retinopathy was statistically significant (P<0.001).

Ethnic Group	Temporal Relationship	Correlation Co-efficient (r)	*P*	R^2 (%)
Indo-Asian	Retinopathy and Hypertension	0.85	<0.001	72
	Hypertension and Proteinuria	0.78	<0.001	61
	Retinopathy and Proteinuria	0.39	N.S.	
Black	Retinopathy and Hypertension	0.90	<0.001	80
	Hypertension and Proteinuria	0.97	<0.001	94
	Retinopathy and Proteinuria	0.78	<0.05	61
White	Retinopathy and Hypertension	0.83	<0.001	69
	Hypertension and Proteinuria	0.84	<0.001	71
	Retinopathy and Proteinuria	0.82	<0.001	79

Level of significance: $P \leq 0.05$ N.S. Not significant R^2: Multiple correlation co-efficient

Table 8.6: Relationship between time to development of retinopathy, hypertension and proteinuria in different ethnic groups

There appeared to be a different relationship between the onset of retinopathy and the other two complications in Indo-Asian patients in comparison to White and Black patients (Table 8.6).

When correlations between various clinical parameters were examined to assess if the relationship between two factors could identify potential risks for specific ethnic groups the following were found: In White patients the only correlation found between the following parameters: age, duration of diabetes, serum creatinine concentration, proteinuria, diastolic and systolic blood pressures, body mass index, HbA1 at referral and over four years was between age and serum creatinine (r=0.40, $P<0.05$). Regression analysis demonstrated that age was 16% predictive of the variance in serum creatinine.

In Indo-Asian patients there was a weak negative correlation between diastolic blood pressure and creatinine (r= -0.49, *P*=0.05) plus a positive correlation between BMI and HbA1 at referral (r=0.67, *P*<0.05) (Figure 8.13).

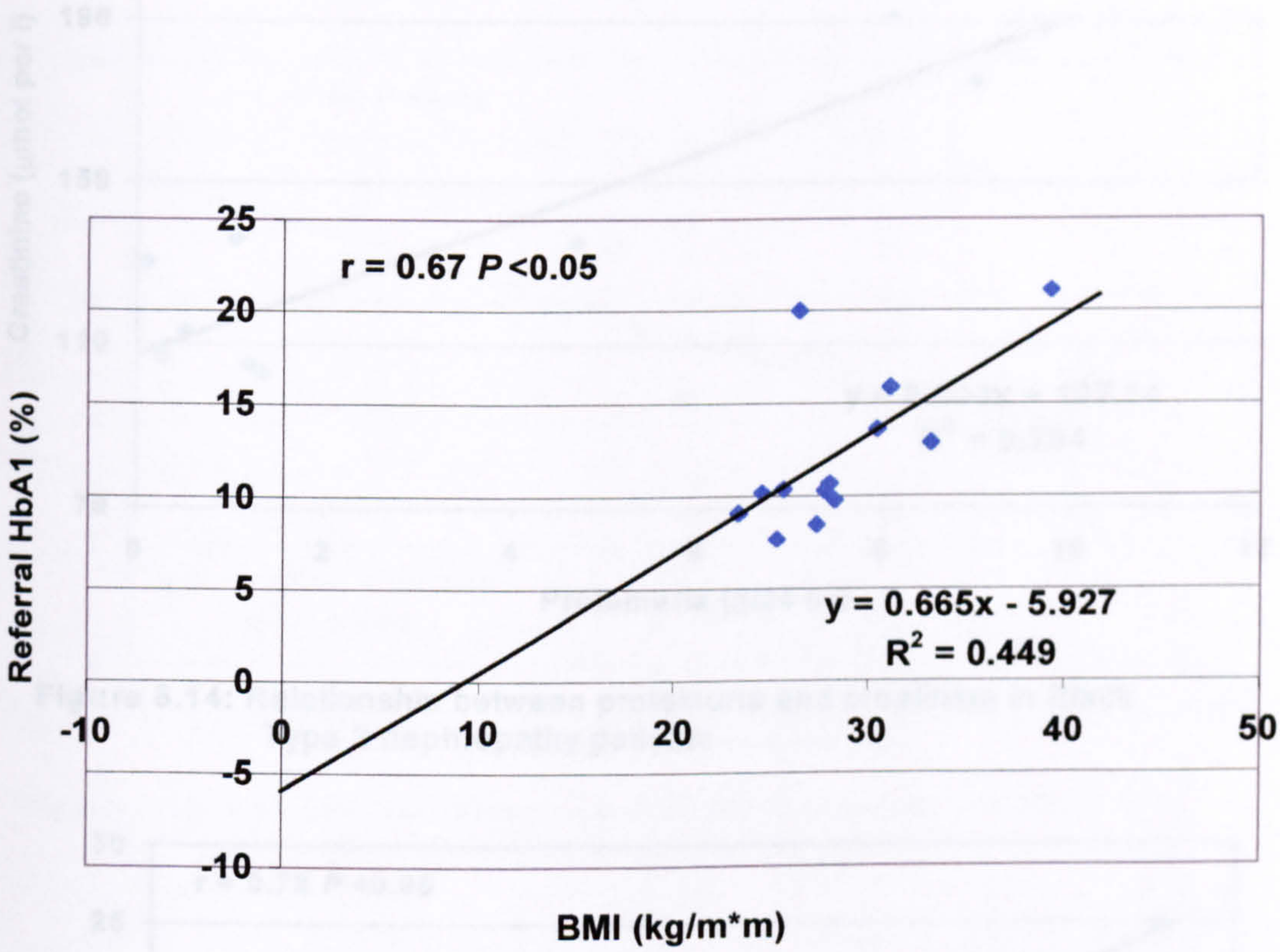


Figure 8.13: Relationship between BMI and HbA1 at referral in Indo-Asian Type 2 patients with nephropathy

In Black patients the correlation between serum creatinine and proteinuria was strong and positive (r=0.89, *P*<0.001) (Figure 8.14) as was the correlation between duration of diabetes and diastolic blood pressure (r=0.78 *P*<0.05) (Figure 8.15).

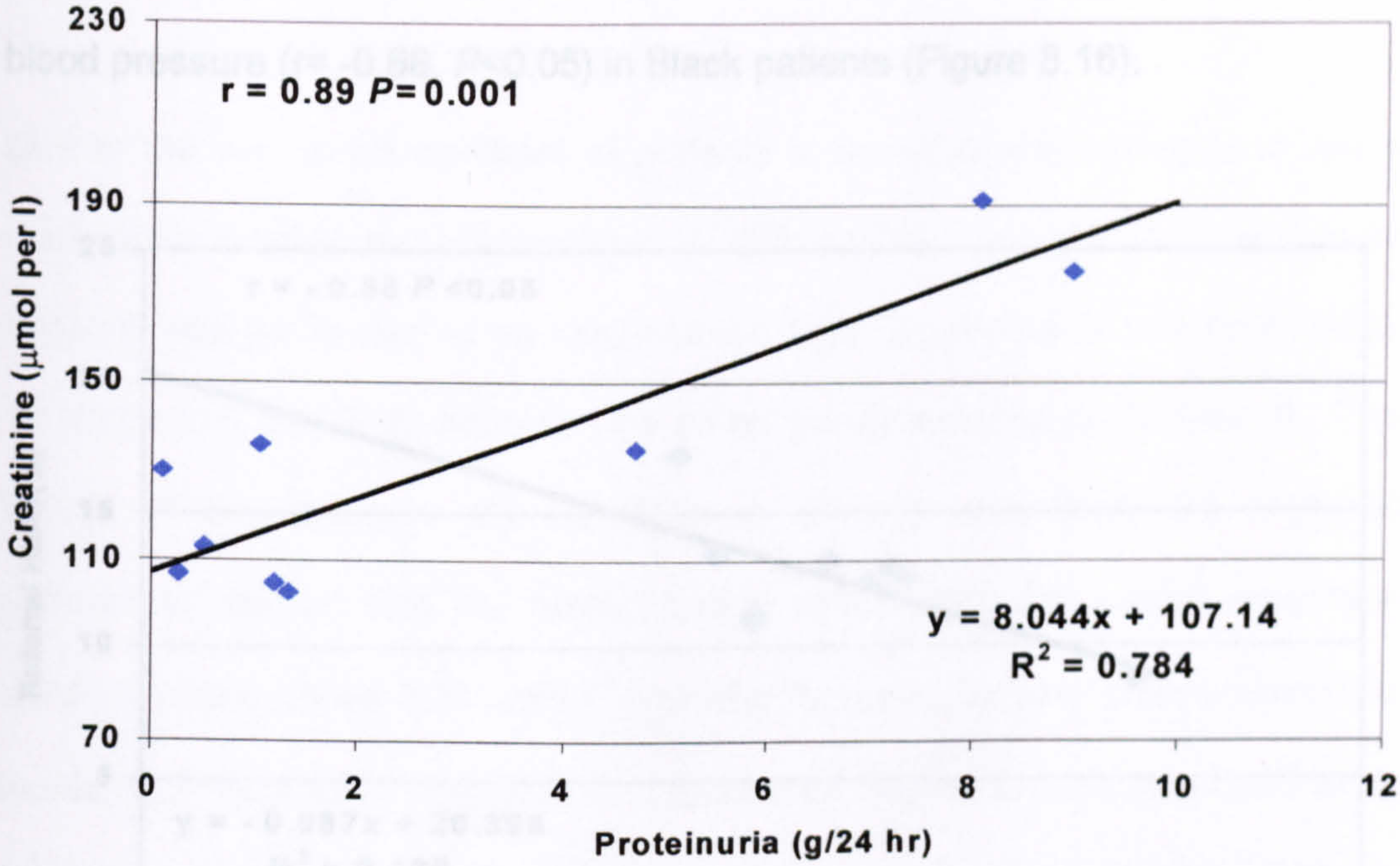


Figure 8.14: Relationship between proteinuria and creatinine in Black Type 2 nephropathy patients

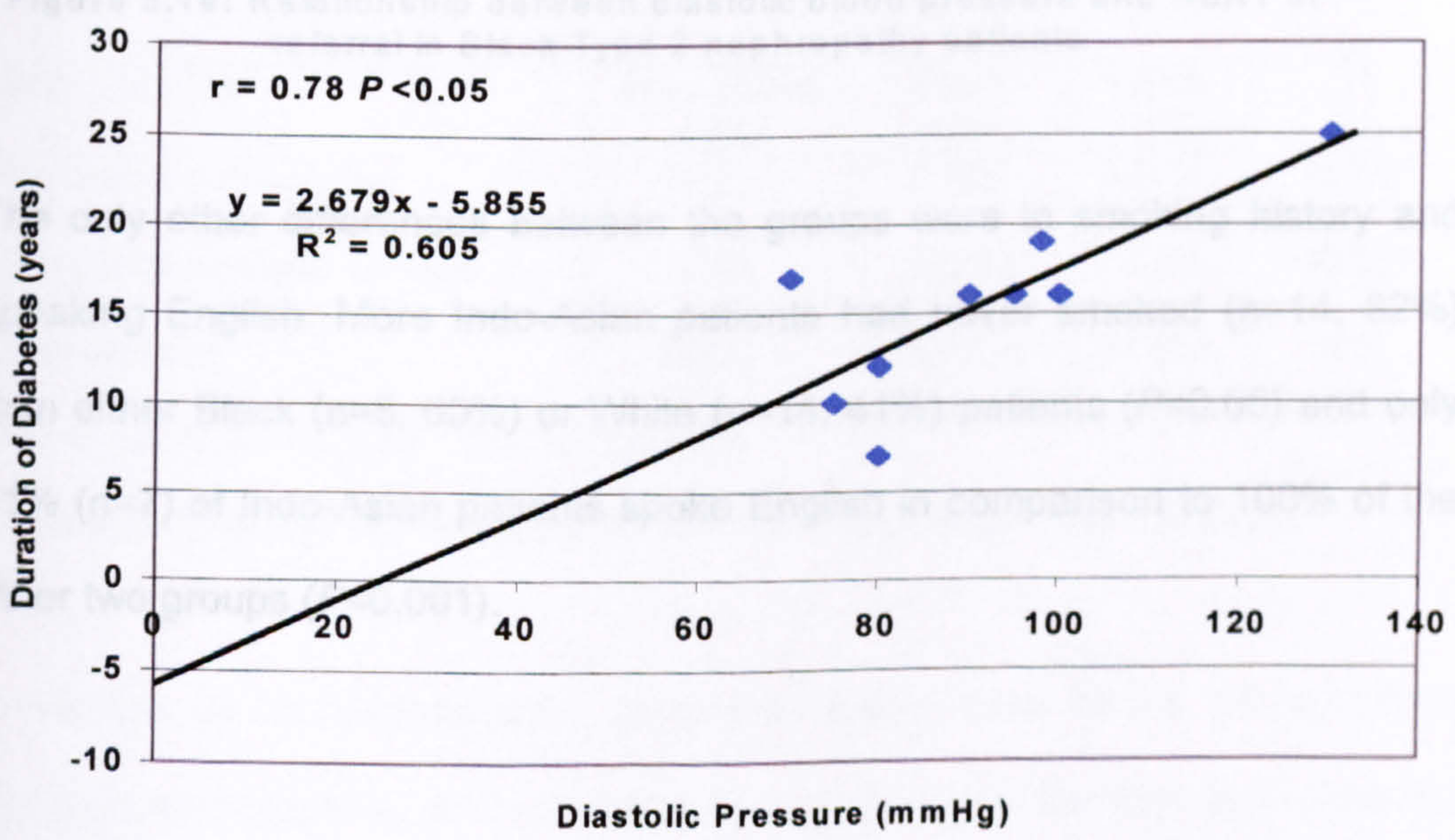


Figure 8.15: Relationship between duration of diabetes and diastolic blood pressure in Black Type 2 nephropathy patients

There was also a negative correlation between HbA1 at referral and diastolic blood pressure (r= -0.68, $P<0.05$) in Black patients (Figure 8.16).

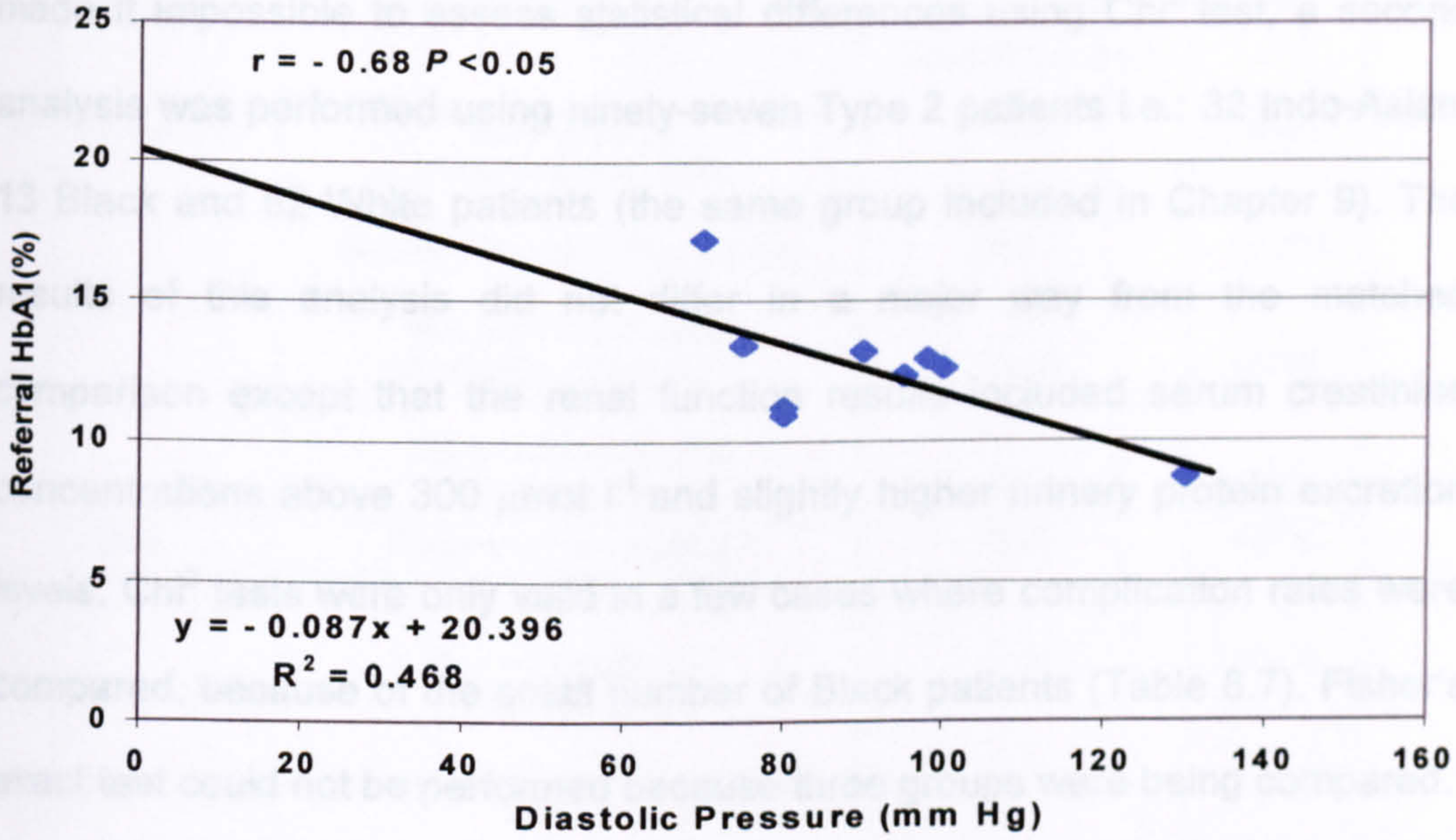


Figure 8.16: Relationship between diastolic blood pressure and HbA1 at referral in Black Type 2 nephropathy patients

The only other differences between the groups were in smoking history and speaking English. More Indo-Asian patients had never smoked (n=14, 82%) than either Black (n=6, 60%) or White (n=14, 41%) patients ($P<0.05$) and only 41% (n=7) of Indo-Asian patients spoke English in comparison to 100% of the other two groups ($P<0.001$).

8.5.5 *Results of Analysis of Larger Numbers of Patients*

Due to the very small numbers of patients in this matched comparison which made it impossible to assess statistical differences using Chi^2 test, a second analysis was performed using ninety-seven Type 2 patients i.e.: 32 Indo-Asian, 13 Black and 52 White patients (the same group included in Chapter 9). The results of this analysis did not differ in a major way from the matched comparison except that the renal function results included serum creatinine concentrations above 300 μmol l^{-1} and slightly higher urinary protein excretion levels. Chi^2 tests were only valid in a few cases where complication rates were compared, because of the small number of Black patients (Table 8.7). Fisher's exact test could not be performed because three groups were being compared.

Complication	Indo-Asian n (%)	Black n (%)	White n (%)	*P*
Peripheral neuropathy	8 (25)	4 (31)	30 (58)	<0.005
Peripheral vascular disease	2 (6)	3 (23)	28 (54)	<0.001
Hypertension	31 (97)	13 (100)	47 (90)	N.S.

Level of significance: $P \leq 0.05$ N.S.: Not significant

Table 8.7: Statistical significance of complications from larger ethnic analysis.

A number of correlations were performed within each ethnic group, which included dichotomous and continuous variables, to identify any potential differences between the groups. There were several correlations, all of which were relatively weak, but were statistically significant. Those variables in which

the numbers were small (< 10) were not included (all correlations in Black patients fitted this category) (Table 8.8).

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Group	Variables	Correlation Co-efficient (r)	*P*
Indo-Asian	Retinopathy / Defaulting from clinic	0.36	<0.05
	Gender / Not speaking English	0.52	<0.005
	Not speaking English /Defaulting from clinic	-0.35	<0.05
	Not speaking English /Diastolic Pressure	0.38	<0.05
White	Peripheral neuropathy / Peripheral vascular disease	0.30	<0.05
	Smoking history / Systolic pressure	-0.23	<0.05
	Smoking history / Hypertension	-0.28	<0.05
	Systolic pressure / Hypertension	0.47	0.001

Level of significance: $P \leq 0.05$

Table 8.8: Correlation between variables in different ethnic groups from larger analysis.

In Indo-Asian patients the significance of not speaking English and defaulting from routine diabetic clinic appointments appeared to be closely linked and also with the presence of retinopathy and level of diastolic blood pressure. Only 41% of Indo-Asian patients in the matched analysis and 46% in the larger analysis spoke English and the majority of those (67%) who defaulted from diabetic clinic visits did not speak English. A history of smoking was linked with raised systolic pressure and hypertension in White patients.

8.6 Discussion

This study, albeit in relatively small numbers of patients, has highlighted some similarities and differences between the three ethnic groups. The only difference in prevalence of macrovascular complications between the groups was the small proportion of Indo-Asian (n=2; 6%) patients with peripheral vascular disease in comparison to White patients (n=28, 54%); this finding has been reported in other studies (Gujral *et al*, 1993; UK Prospective Diabetes Study Group, 1994). Similarly, there was a higher prevalence of peripheral neuropathy in White patients compared to both the Indo-Asian and Black groups.

Although the prevalence of hypertension was similar in all of the groups, a larger proportion of Indo-Asian patients were receiving anti-hypertensive medication in comparison to the other two groups. Of those patients with treated hypertension, fewer White (27%) received a combination of different anti-hypertensive medications but still had the same mean blood pressure as Indo-Asian and Black patients, of whom 69% and 60% respectively were on combination therapy. Therefore, this may indicate that (a) Indo-Asian and Black patients had hypertension which may be more difficult to control (b) the medical staff were more open to prescribing combination therapy to these two groups, or (c) that the high prevalence of peripheral vascular disease in White patients

meant that reducing blood pressure levels in this group would have compromised the patients' peripheral vasculature.

The statistically significant higher mean systolic pressure in patients with treated hypertension in the Indo-Asian group in comparison to untreated patients may be reflective of clinicians concentrating on treating diastolic hypertension and an acceptance of systolic pressures above the upper limit of normal except in cases where systolic pressures were considerably raised. The finding that patients in all three groups with treated hypertension were heavier than those with untreated hypertension not only suggests that being overweight contributes to hypertension but that clinicians were more open to prescribing anti-hypertensive therapy for larger patients. In White patients although the correlation between hypertension and smoking history was not strong it was significant especially as there was also a correlation between systolic pressure and a history of smoking in this same group. An Italian study of Type 2 patients with micro- and macroalbuminuria has demonstrated an increased risk of developing nephropathy associated with ex-smoking and this has been confirmed herein (Chapter 6) (Bruno *et al,* 1996). The EURODIAB study has shown that Type 1 patients who were ex-smokers had a higher prevalence of macroalbuminuria and proliferative retinopathy than current or non-smokers (Chaturvedi *et al*, 1995). The long term effects on both large and small blood vessels of diabetic patients having smoked at some point in their lives appear

to have a cumulative effect in people who are susceptible to developing nephropathy.

The shorter duration of diabetes until onset of retinopathy, hypertension and proteinuria found in Indo-Asian patients suggests that this group may be developing these complications at an earlier stage of their diabetes than either Black or White patients. However, it is worth remembering that in Type 2 diabetes the exact onset of diabetes is never actually known and diagnosis can occur some considerable time after the disease has developed, a finding confirmed by the presence of diabetic complications at diagnosis of diabetes in 2% of Type 2 patients entered into the UK Prospective Diabetes Study (UK Prospective Diabetes Study Group, 1994).

The Indo-Asian group was the only group where there was an absence of a statistically significant correlation between the times of onset of retinopathy and proteinuria although there were strong correlations between the times to development of hypertension and proteinuria and of hypertension and retinopathy. In addition, Indo-Asian patients developed retinopathy at a significantly earlier duration of diabetes than the other two groups. These findings suggests that in Indo-Asian patients there was a different pattern in the development process of the three complications in relation to each other which was not obvious in the other two groups. One explanation of this may be that hypertension may be the link between retinopathy and proteinuria. Klein and

co-workers have demonstrated that systolic blood pressure predicts the incidence of retinopathy and diastolic pressure predicts the progression of retinopathy and it has been tentatively suggested that treatment with ACEI may decrease the progression of retinopathy in patients with normal blood pressures (Klein *et al*, 1989; Chaturvedi *et al*, 1998).

The positive correlation between increasing age and serum creatinine in White patients was due to this group being older than the other two groups (difference in mean and median of 4-5 years, with a wider range) as renal function deteriorates with increasing age. In Indo-Asian patients the positive correlation between increasing BMI and HbA1 at referral was to be expected in that, the more overweight a patient is the more difficult it is to control blood glucose levels. The two correlations with diastolic blood pressure found in Black patients were interesting: Increasing duration of diabetes correlated with increasing blood pressure and decreasing HbA1 at referral correlated with increasing blood pressure. All of these may be related, Black patients with longer duration of diabetes and raised diastolic pressures who developed proteinuria may have received aggressive medical care in the diabetic clinic prior to referral which included addressing poor glycaemic control. However, the numbers in this group were too small for any substantial conclusions to be drawn.

Less than 50% of Indo-Asian patients in this study spoke English. This percentage is higher than has been previously reported in Wolverhampton, where it was found that only 26% of Indo-Asian patients attending diabetes education sessions, presented in Punjabi, spoke English (Wilson *et al*, 1993). However, a survey of Asian diabetic patients in Nottingham showed that 70% spoke English, therefore, the difficulty in communication with health care professionals may not be as widespread across the U.K. as the Wolverhampton figures suggest (Hawthorne, 1990). The weak but statistically significant correlations between not speaking English, defaulting from clinic visits and diastolic pressure cannot be ignored. Being unable to communicate with healthcare professionals may directly influence the patient's understanding of the importance of regularly attending the diabetic clinic. Previous research in Wolverhampton has demonstrated that patients who defaulted from routine diabetic clinic follow up have less contact with physicians and more complications than those who attended on a regular basis (Hammersley *et al*, 1985). The association between not speaking English, defaulting from clinic and diastolic blood pressure would appear to be downward spiral for Indo-Asian patients which needs to be broken. Therefore, the inability to speak English, which can prevent patients understanding their disease and how to manage it and which also appears to contribute to patients defaulting from clinic visits, must be regarded as an avoidable risk factor for diabetic complications including nephropathy.

The small number of patients in the analysis of ethnic groups has provided a view of the possible differences between the three groups but in order to statistically prove that there are differences, a much larger study would be needed to increase the power of the study and which would need to include a larger group of Black patients (Silman, 1995; Gordis, 1996).

8.7 Conclusions

1 Retinopathy, hypertension and proteinuria developed earlier in Indo-Asian patients than in either of the other two groups. Therefore, accept H_1 (1): There were differences in presentation of nephropathy between the groups.

2 White patients had a higher prevalence of peripheral neuropathy and peripheral vascular disease than the other two groups. Therefore accept H_1 (2): There were differences in the prevalence of diabetic complications between White, Indo-Asian and Black Type 2 diabetic patients.

3 Fewer White patients with hypertension were receiving anti-hypertensive medication than either Indo-Asian or Black patients.

4 The inability to speak English found in Indo-Asian patients was a risk factor for development of nephropathy and was linked to defaulting from routine clinic visits: Therefore accept H_1 (3): There were risk factors for nephropathy specific to Indo-Asian patients.

5 In White patients age at referral was predictive of serum creatinine concentrations. In the other two groups there were no factors which were

associated with nephropathy that were specific to either ethnic group. Therefore, accept H_1 (4): There were factors associated with nephropathy specific to either White, Indo-Asian or Black diabetic Type 2 patients.

Chapter 9

Evaluation of the Effectiveness of Routine Nephrological Out-Patient Clinical Care on Survival and Assessment of Factors Which May Influence Survival in Patients with Diabetic Nephropathy

9.1 Introduction

Both the Diabetes Control and Complications Trial in Type 1 diabetes and the United Kingdom Prospective Diabetes Study in Type 2 patients (both studies with large numbers of patients) have confirmed that diabetic nephropathy can be delayed by tight glycaemic control (Diabetes Control and Complications Trial Research Group, 1993; Turner *et al*, 1996). In 1982, Mogensen followed by Parving and colleagues (1983) demonstrated in studies with small numbers of patients (n=6 and n=12 respectively) that control of hypertension also prevented the progression of nephropathy, a finding that has been confirmed subsequently (Mogensen, 1982; Parving *et al*, 1983; The EUCLID Study Group, 1997). Despite this knowledge, diabetic nephropathy is increasingly a major cause of end stage renal disease and death. Within a five year period, the percentage of patients with diabetes who were receiving renal replacement therapy in Heidelberg in Germany had increased from 36% to 59% (Ritz *et al*, 1999). In other countries the percentage of deaths from end stage renal failure (ESRF) secondary to nephropathy is high e.g.: in Japan, 33% of cases of diabetes diagnosed over a 14 year period and in Missouri, USA, 54% of Type 2 in comparison to 10% of Type 1 patients died from ESRF (Diabetes Epidemiology Research International Mortality Study Group, 1991; Hellman *et al*, 1997). Once persistent proteinuria is established prognosis is poor: In two Danish studies 35% of patients with macroalbuminuria died within five years and 44% of patients with overt nephropathy died within ten years (Parving, 1999). It is

worth noting that in 1989, Parving and Hommel demonstrated that anti-hypertensive treatment in nephropathy patients reduced the cumulative death rate from ESRF over ten years to 18% in comparison to much higher rates in previous studies (Parving and Hommel, 1989).

There are also reported differences in the characteristics of nephropathy in various ethnic groups including Indo-Asian and Black Type 2 patients who have increased prevalence and incidence of ESRF due to nephropathy and Pima Indians with nephropathy who have increased mortality rates from ESRF and cardiovascular disease (Nelson *et al*, 1988; Cowie *et al*, 1989; Gujral *et al*, 1997). Similarly, there is a high incidence of nephropathy in Japanese Type 2 patients with early-onset of diabetes (Yokoyama *et al*, 1998). While comparison of Type 1 patients in Europe and the USA has shown an increased prevalence of nephropathy in the USA (Lloyd *et al*, 1996).

This study is designed to evaluate whether routine follow up of nephropathy patients in a nephrology clinic affects rates of survival and whether there are differences in factors which influence survival in both types of diabetes and in different ethnic groups.

9.2 Objectives

1 To determine whether routine follow up of patients in a nephrology clinic affects survival of diabetic patients with nephropathy.

2 To determine whether different factors affect survival in Type 1 and Type 2 nephropathy patients.

3 To determine whether different factors affect survival in different ethnic groups.

9.3 Hypotheses

1 H_0: Routine follow up in a nephrology clinic did not improve survival rates in Type 1 and Type 2 diabetic nephropathy patients

H_1: Routine follow up in a nephrology clinic did improve survival rates in Type 1 and Type 2 diabetic nephropathy patients

2 H_0: Survival time was the same in patients from different ethnic groups with diabetic nephropathy.

H_1: Survival time was not the same in patients from different ethnic groups with diabetic nephropathy.

3 H_0: There was no difference in factors which affect survival in Type 1 and 2 diabetic patients with nephropathy.

H_1: There was a difference in factors which affect survival in Type 1 and 2 diabetic patients with nephropathy.

4 H_0: There was no difference in factors which affect survival in different ethnic groups.

H_1: There was a difference in factors which affect survival in different ethnic groups

9.4 Methods

Diabetic patients referred for nephrological assessment were followed up over a maximum period of twelve years (1987-1998) to assess the effect of normal clinical practice on survival. Normal clinical practice was defined as treatment determined by consultant nephrologists for individual patients and individual medical problems on an "intention to treat" basis; there was no written pre-determined protocol for treatment which could be followed by junior medical staff, although individual cases were discussed with the consultant. Follow up was defined as regular attendance at the out-patient clinic, though the number of visits per year varied according to the clinical condition of the patient. Patients were excluded from the study if they were receiving renal replacement therapy at the study onset or if they had only one functioning kidney, as this may have affected the rate of decline of renal function. As patients were referred at different times over the twelve year period, length of follow up time varied from patient to patient.

Data were collected from patients' hospital records on blood pressure measurements, serum creatinine concentrations, urinary protein levels, and glycaemic control as measured by HbA1 (both pre and post-referral), over the time period since referral, as well as demographic data. Information on end points was also collected. The end points were: survival without reaching ESRF, need for renal replacement therapy (RRT), survival on RRT, death,

death following RRT and lost to follow up. Cumulative survival, using Kaplin-Meier curves and the Log rank test were used to compare equality of survival in Type 1 and Type 2 patients and in different ethnic groups. Cause of death was also recorded. In some cases, the causes of death could not be determined due to inability to locate patients' hospital records after death. Data collected at referral were the baseline data used to compare data collected annually in subsequent years up to year 7, from this time point numbers of patients were too low to reliably perform statistical analysis.

A number of different parameters were examined to find the potential effect of these on survival. Hypertension was defined as a systolic pressure > 140 mm Hg or diastolic pressure > 90 mm Hg and this decided the "need for treatment". Age at renal referral, duration of diabetes, time from onset of overt proteinuria to renal referral, duration of diabetes until onset of proteinuria, systolic and diastolic blood pressures, serum creatinine, 24 hour urinary protein excretion, anti-hypertensive treatment (as measured by point scale, see Appendix 1) and presence or absence of this at baseline. All of the above were compared in all of the Type 1 and Type 2 groups and, in addition, two other analyses were performed: 1) in three ethnic groups: Indo-Asian (people with ethnic origins in the Indian subcontinent whether born there or elsewhere): Black (people with ethnic origins in either Africa or the Caribbean, irrespective of place of birth): and White patients and 2) a subgroup analysis of Type 1 and Type 2 patients with serum creatinine levels below 120 μmol l^{-1} (upper limit of normal) to

remove bias due to advanced renal disease. Cox regression analysis was performed and the Cox proportional hazard model was used to consider the above parameters in terms of cumulative survival and to determine hazard ratios for specific parameters. As the numbers of patients were small, a limit of three parameters was used together as covariates.

Numerical data were analysed using Student t-test, categorical data were analysed using Chi^2 test and Fisher's Exact Test when expected frequencies were less than 5. Analysis of variance was used to determine differences within groups of repeated measures and between the groups. Standard deviation (SD), standard error (SE) and 95% confidence intervals (CI) were used in Kaplin-Meier analysis, SE were not calculated if the number of deaths was less than the number of censored data. The level of significance was $P<0.05$. As the number of patients in the various groups analysed decreased to less than ten over the years of follow up, statistical analysis was only performed, if number of patients was greater than 10, except when Fisher's Exact Test was used.

9.5 Results

9.5.1 Demographic Data

From the original cohort of 47 Type 1 patients with nephropathy, three were excluded as they were receiving renal replacement therapy at the onset of the study. Forty-four Type 1 and 97 Type 2 patients were included; five Type 2 patients with only one functioning kidney were excluded.

There was no significant difference in gender ratio between the two groups, however, as expected, the Type 2 group had more Indo-Asian and Black patients than the Type 1 group ($P<0.05$) (Table 9.1).

Demographics	Type 1 n (%)	Type 2 n (%)	*P*
TOTAL	44 (100)	97 (100)	
GENDER			
Male	32 (73)	65 (67)	N.S.
Female	12 (27)	32 (33)	N.S.
ETHNIC GROUP			
Asian	5 (11)	32 (33)	
Black	5 (11)	13 (13)	<0.05
White	34(78)	52 (54)	
DURATION OF DM (years)			
Range	8-42	0-11	
Mean (±SD)	23 (±8)	13 (±7)	<0.001
Median	23	14	
AGE AT REFERRAL (YEARS)			
RANGE	18-73	39-83	
MEAN (±SD)	44 (±13)	61(±9)	<0.001
MEDIAN	45	62	

DM: Diabetes mellitus SD: Standard deviation N.S.: Not significant

Table 9.1: Demographic data on nephropathy patients followed over an eleven year period.

Mean duration of diabetes at nephrological referral was ten years shorter in the Type 2 group than in Type 1 patients ($P<0.001$). The mean age of the Type 2 group was 17 years older than that of Type 1 patients ($P<0.001$). The mean (Type 1: 3 years; Type 2: 2 years) and median (1 year in both groups) time to nephrological assessment after the second proteinuria-positive dipstick test was similar in both groups (P=N.S.). However, there was a statistically significant difference between the groups in the time from onset of proteinuria to nephrological referral with Type 2 patients being referred more quickly [range: 0-11 years; mean: 1.6 (SE$\pm$ 0.2) years] than the Type 1 group [range: 0-29 years; mean: 3.3 (SE$\pm$0.8) years] ($P<0.05$).

Twenty three (52%) of the Type 1 group in comparison to 25 (26%) of Type 2 patients were alive at the end of the study while 3 (7%) of Type 1 and 13 (13%) of Type 2 patients were lost to follow up ($P<0.01$) (Table 9.2).

End Points	Type 1 n (%)	Type 2 n (%)	*P*
TOTALS:	44 (100)	97(100)	
Alive	23 (52)	25 (26)	
Dead	18 (41)	59 (61)	<0.01
Lost to Follow Up	3 (7)	13 (13)	
SUBTOTALS:			
Patients on RRT:			
Alive	8 (18)	3 (3)	
Dead	7 (16)	13 (13)	
Patients not on RRT:			
Alive	15 (34)	22 (22)	
Dead	11 (25)	46 (47)	<0.005

RRT: Renal replacement therapy

Table 9.2: End points reached by nephropathy patients after an eleven-year period.

Three of the Type 1 group were alive after 11 years of follow up and three Type 2 patients after ten years (Figure 9.1). Fifteen (34%) Type 1 patients and 16 (16%) of the Type 2 group had received renal replacement therapy (RRT). There was no significant difference in mean time to starting RRT between the groups: Type 1: 3(SD±3) years and Type 2: 2(SD±2) years (*P*=N.S.). Seven Type 1 and thirteen Type 2 patients on RRT had died (*P*<0.005) (Table 9.2). One Type 1 and seven Type 2 patients had died from ESRF without receiving RRT within a short time of referral (Type 1 time to death: 2 years and in Type 2: mean: 1(SD±1) year).

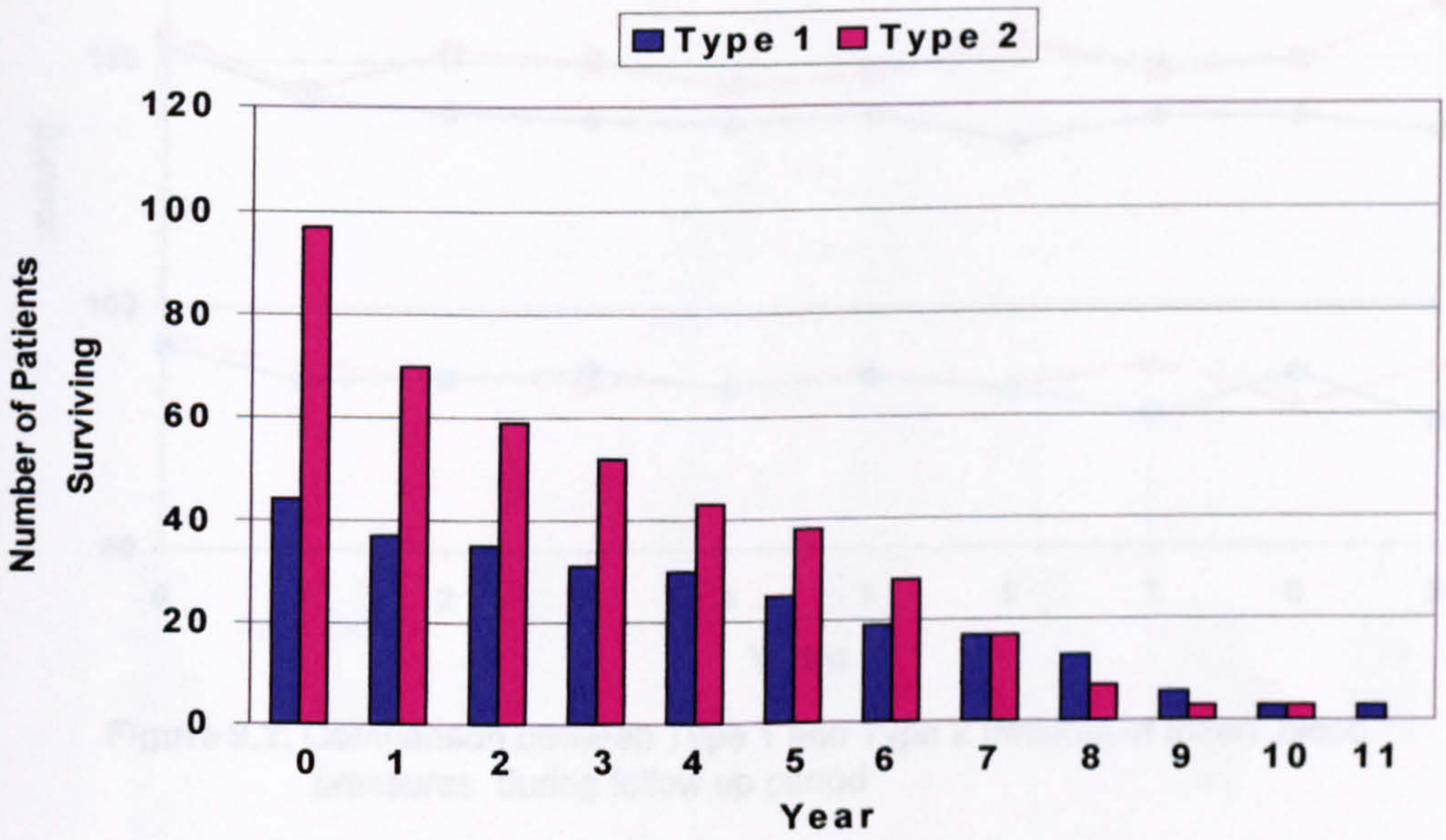

Figure 9.1: Numbers of patients still alive at the end of each year of follow up

Comparison of systolic blood pressure recorded annually showed that there was no statistical difference in systolic pressure between the groups at baseline [Type 1; mean: 164 (SD±28) mm Hg versus Type 2; mean: 167 (SD±27)mm Hg]. Systolic pressures compared between baseline and year 1, had decreased in both groups (P<0.001). There were significant differences in systolic pressure between the groups in years 2, 3 and 6 (P <0.05 in all three years) (Figure 9.2).

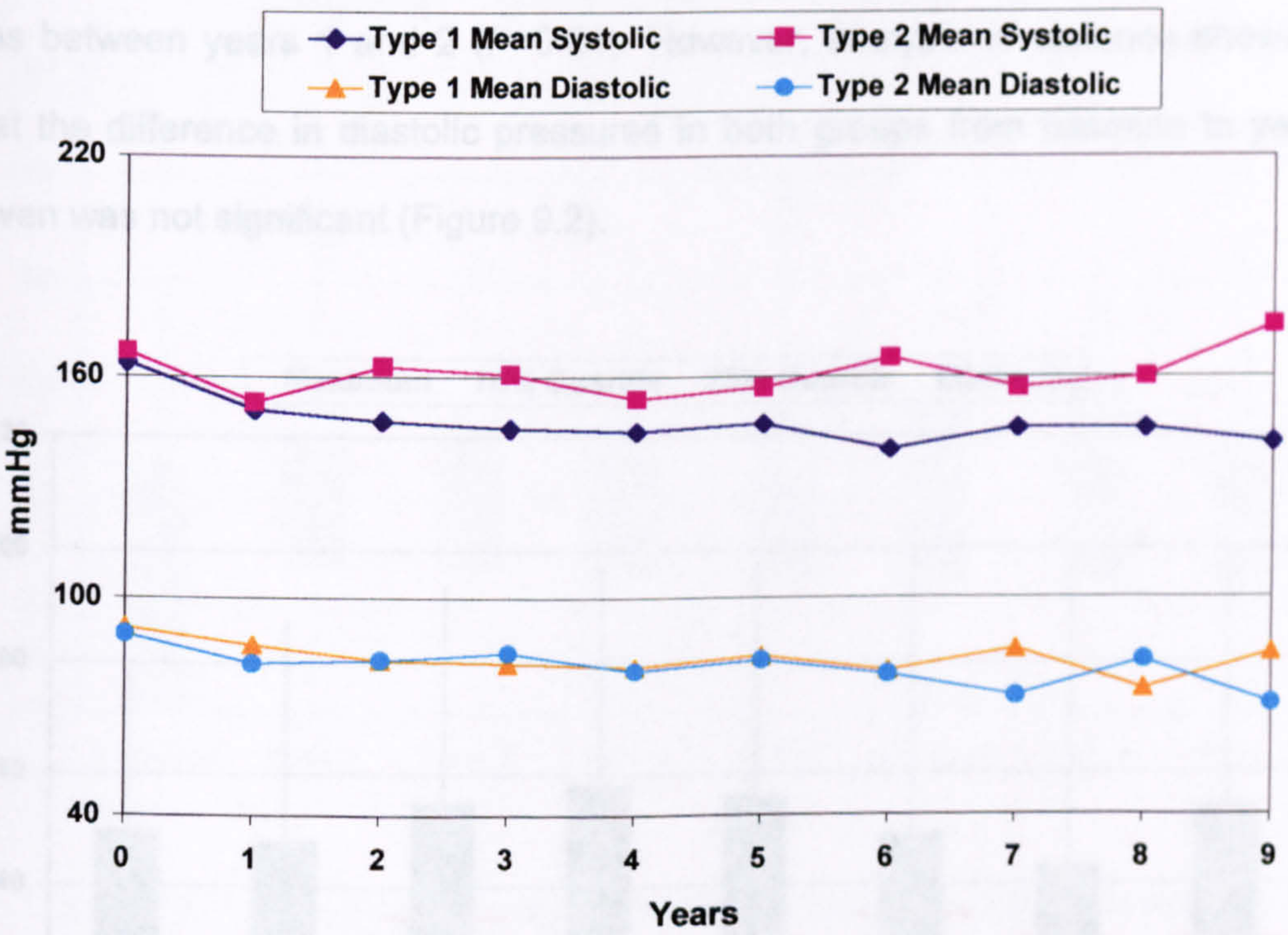

Figure 9.2: Comparison between Type 1 and Type 2 patients of mean blood pressures during follow up period

From year two onwards, Type 2 patients had higher systolic pressures than the Type 1 group. However, analysis of variance showed that there was no

significant difference over seven years of follow up, in either group, in systolic pressure from baseline to year seven (Figure 9.2).

There was no difference in diastolic pressures between the groups at baseline [Type 1; mean: 92 (SD± 12) mm Hg versus Type 2; mean: 90 (SD±17) mm Hg]. However, by the end of year 1 diastolic pressures in both groups had decreased and the difference from baseline was significant ($P<0.005$), as it was between years 1 and 2 ($P<0.01$). However, analysis of variance showed that the difference in diastolic pressures in both groups from baseline to year seven was not significant (Figure 9.2).

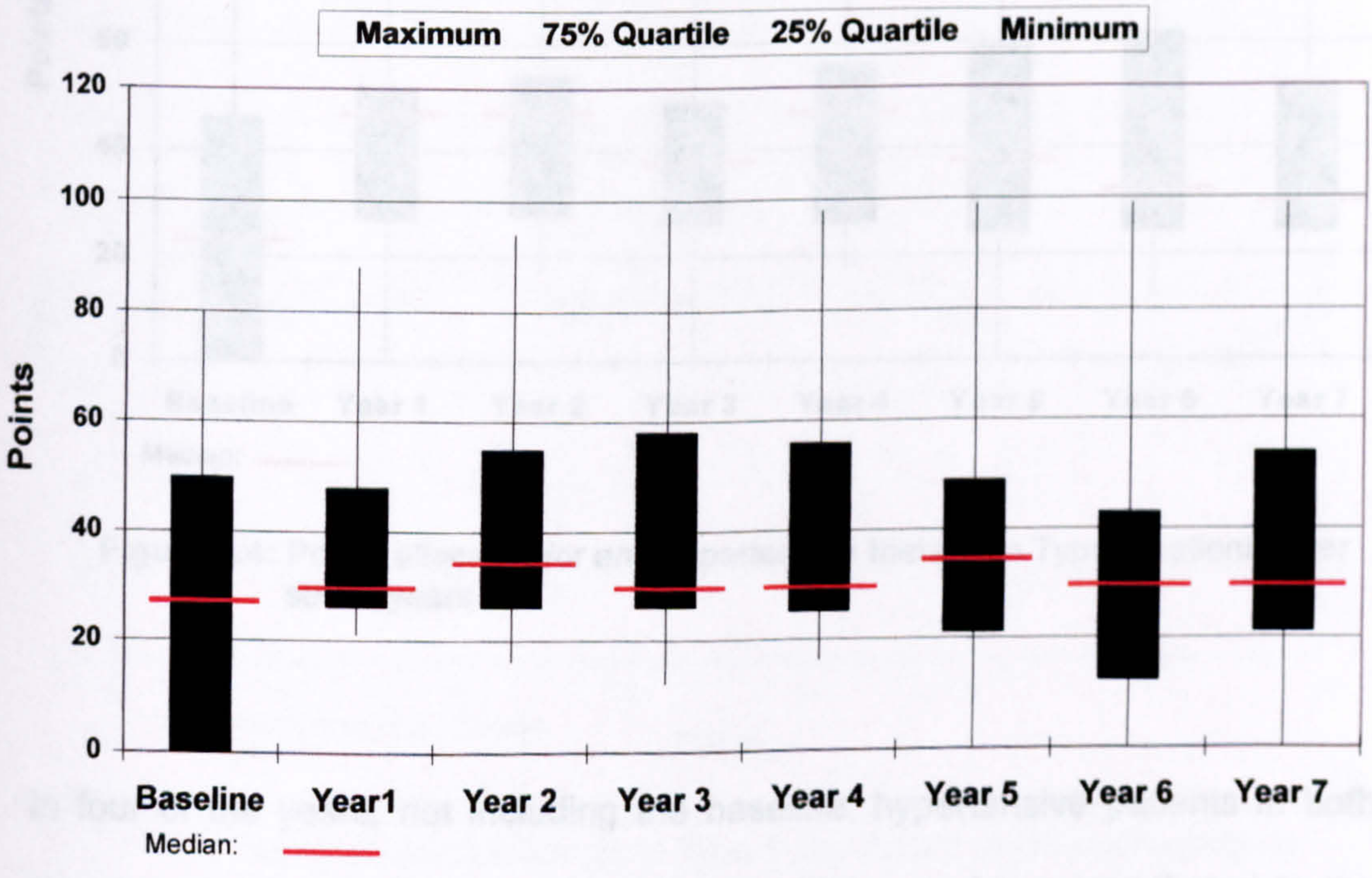

Figure 9.3: Points allocated for anti-hypertensive therapy in Type 1 patients over seven years

Thirty nine (86%) Type 1 and 87 (90%) of Type 2 patients were hypertensive at referral (*P*=N.S.) Thirteen (33%) Type 1 patients and 46 (55%) of Type 2 hypertensive patients were not receiving anti-hypertensive therapy (*P*<0.05). Over seven years there was a wide range for points (Appendix 1) for anti-hypertensive treatment in both groups of patients with median values frequently towards the bottom of the range in Type 1 patients (Figure 9.3 and 9.4).

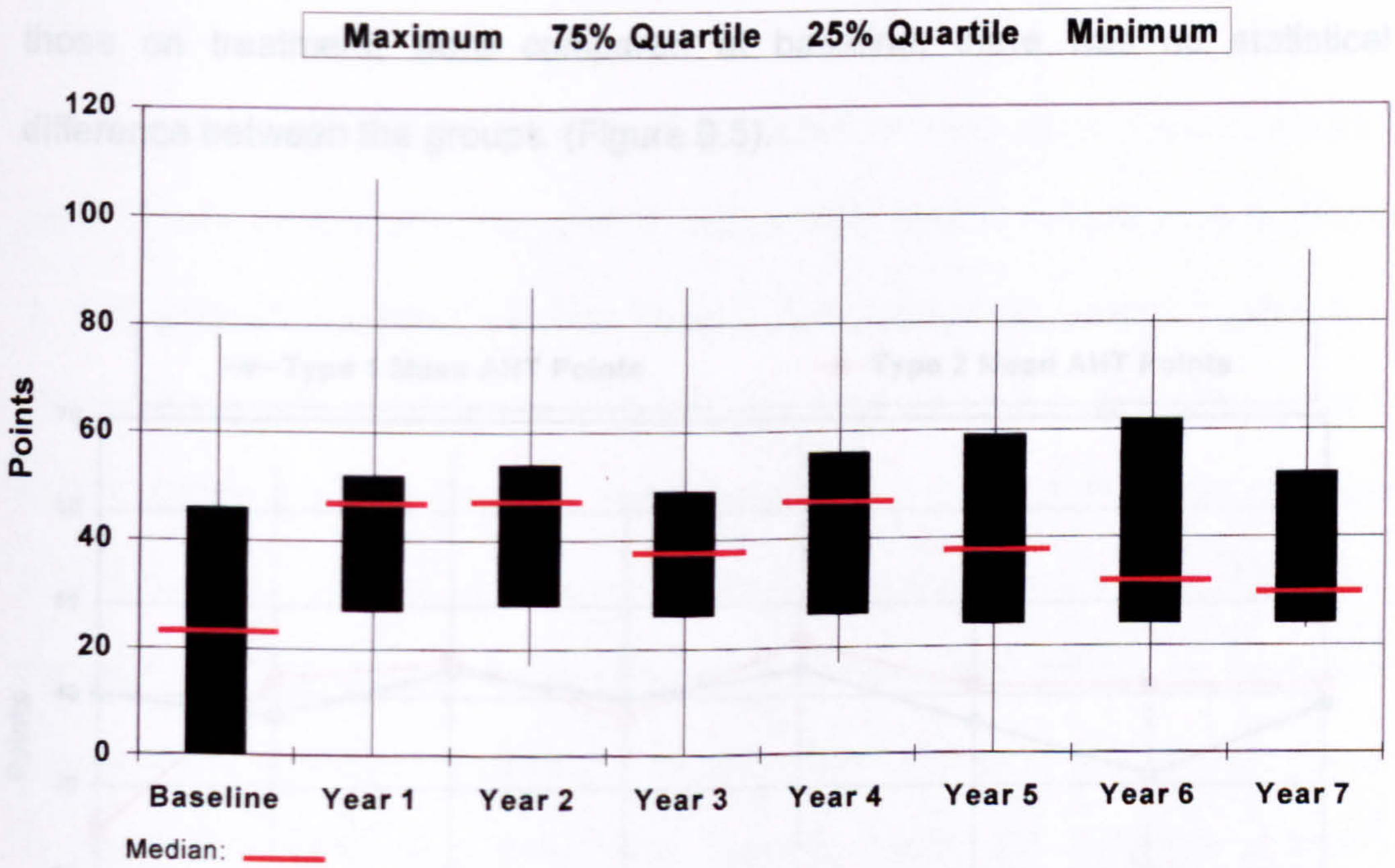

Figure 9.4: Points allocated for anti-hypertensive therapy in Type 2 patients over seven years

In four of the years, not including the baseline, hypertensive patients in both groups were not receiving drug treatment, which may have contributed to the persistent systolic hypertension observed in Type 2 patients. Type 1 patients

received more anti-hypertensive treatment at baseline than the Type 2 group. There was no difference between the groups in type of anti-hypertensive drugs used in treatment (*P* =N.S.). Fifteen (38%) of Type 1 and 15 (17%) of Type 2 patients were on ACEI. Multiple anti-hypertensive therapy (more than one type of drug) was being used in the same percentages of both groups (duo therapy: 21% and triple therapy: 8%) except for 2% of Type 2 patients who required quadruple therapy. However, when points for anti-hypertensive treatment, for those on treatment, were compared at baseline, there was no statistical difference between the groups (Figure 9.5).

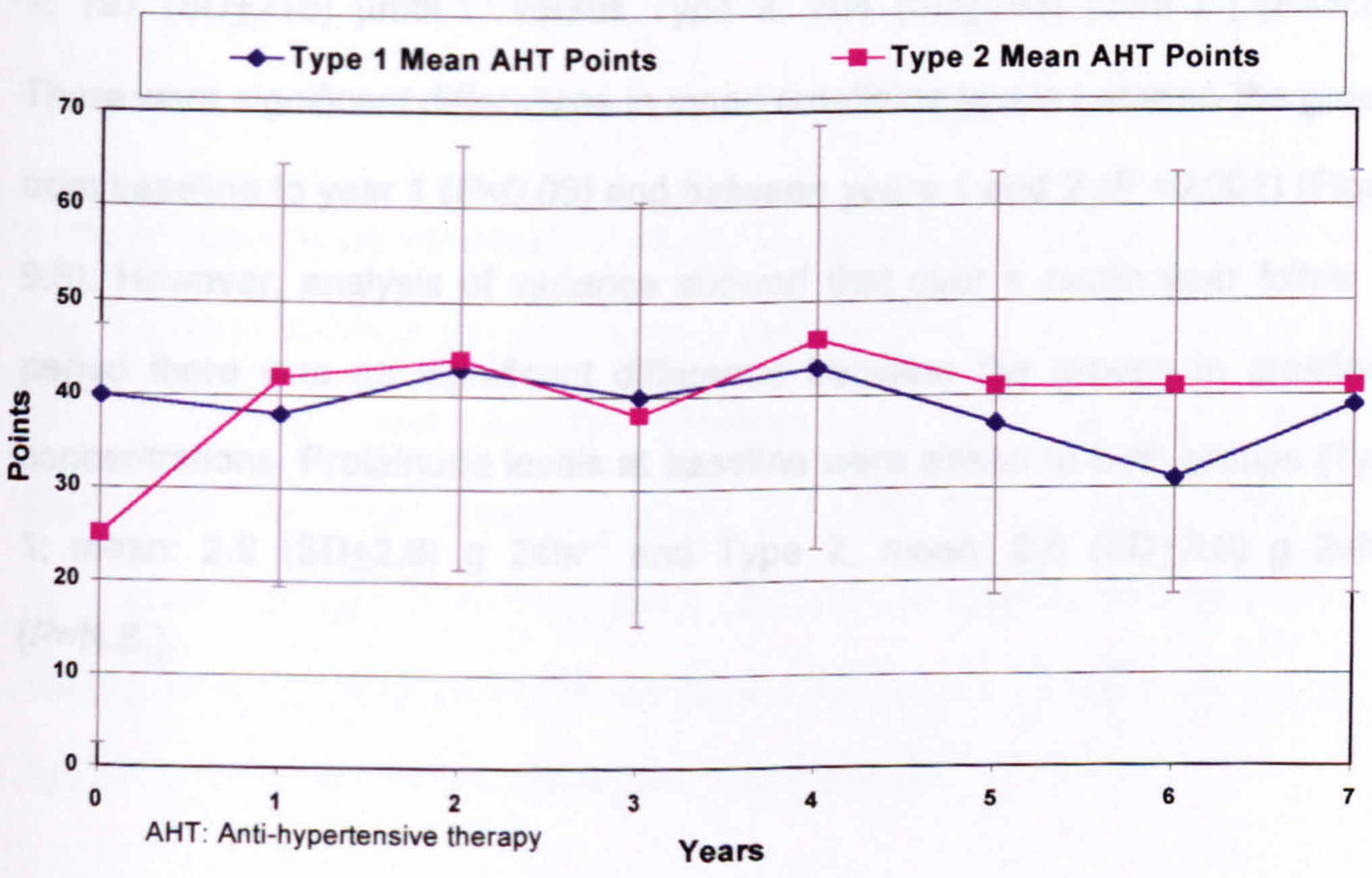

Figure 9.5: Comparison of mean points allocated for anti-hypertensive therapy over seven years of follow up in Type 1 and Type 2 nephropathy patients

The difference in anti-hypertensive points between baseline and year one, which was statistically significant, demonstrated that the number of patients receiving anti-hypertensive treatment had increased in both groups (P<0.001) (Figure 9.5). However, there was no difference between baseline and subsequent years when all seven years were added (P=N.S.) (Figure 9.5). Type 2 patients had a higher overall mean point rating for anti-hypertensive treatment for the majority of the seven years than the Type 1 group.

The ranges of serum creatinine levels at baseline were wide: Type 1: 65-858 μmol l^{-1} and Type 2: 65-1140 μmol l^{-1} with similar means in both groups: [Type 1: 197 (SD$\pm$215) μmol l^{-1} versus Type 2: 204 (SD$\pm$193) μmol l^{-1}] (P=N.S.). There were significant differences in mean creatinine levels between the groups from baseline to year 1 (P<0.05) and between years 1 and 2 (P <0.001) (Figure 9.6). However, analysis of variance showed that over a seven year follow up period there was no significant difference between the groups in creatinine concentrations. Proteinuria levels at baseline were similar in both groups (Type 1; mean: 2.9 (SD$\pm$2.6) g $24hr^{-1}$ and Type 2; mean: 2.8 (SD$\pm$3.6) g $24hr^{-1}$ (P=N.S.).

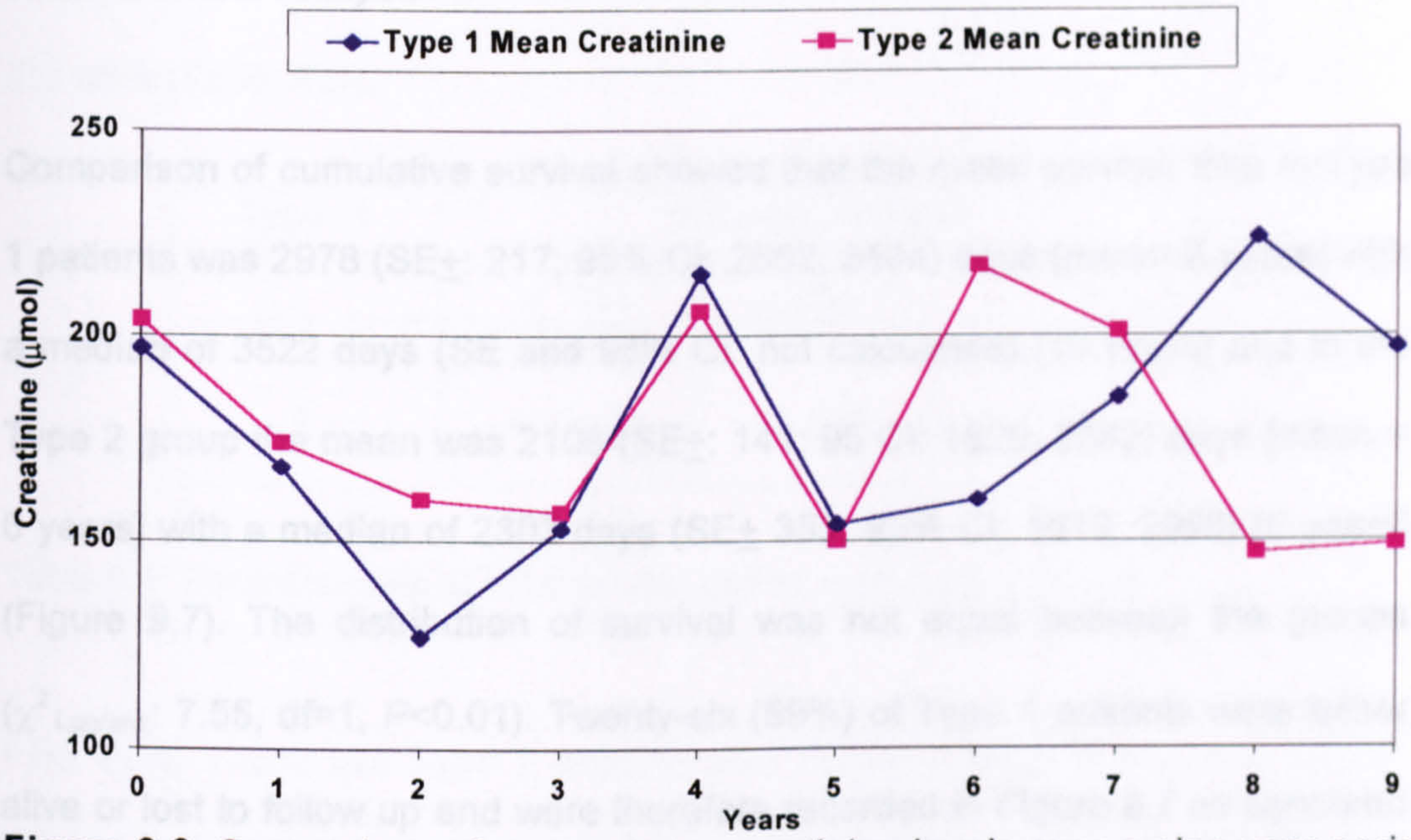

Figure 9.6: Comparison of mean serum creatinine levels over a nine year period: Type 1 versus Type 2 patients

In those patients where cause of death was known, congestive cardiac failure was the most common cause in Type 1 patients (n=4; 22%) followed by ESRF (n=3; 17%) and cerebrovascular accidents (n=3; 17%). In Type 2 patients, ESRF was the most common cause of death (n=14; 24%) followed by myocardial infarction (n=8; 14%) and 4 (7%) died of cerebrovascular accidents. The cause of death could not be ascertained in 4 (22%) Type 1 and 27 (46%) Type 2 patients making statistical analysis unreliable.

9.5.2 *Survival Analysis*

Comparison of cumulative survival showed that the mean survival time in Type 1 patients was 2978 (SE±: 217; 95% CI: 2552, 3404) days [mean=8 years] with a median of 3522 days (SE and 95% CI. not calculated) [10 years] and in the Type 2 group the mean was 2106 (SE±: 141; 95 CI: 1829, 2382) days [mean = 6 years] with a median of 2303 days (SE± 353; 95% CI: 1610, 2996) [6 years] (Figure 9.7). The distribution of survival was not equal between the groups ($\chi^2_{Logrank}$: 7.55, df=1, $P<0.01$). Twenty-six (59%) of Type 1 patients were either alive or lost to follow up and were therefore recorded in Figure 9.7 as censored (marked as a +) as were 38 (39%) of Type 2 patients.

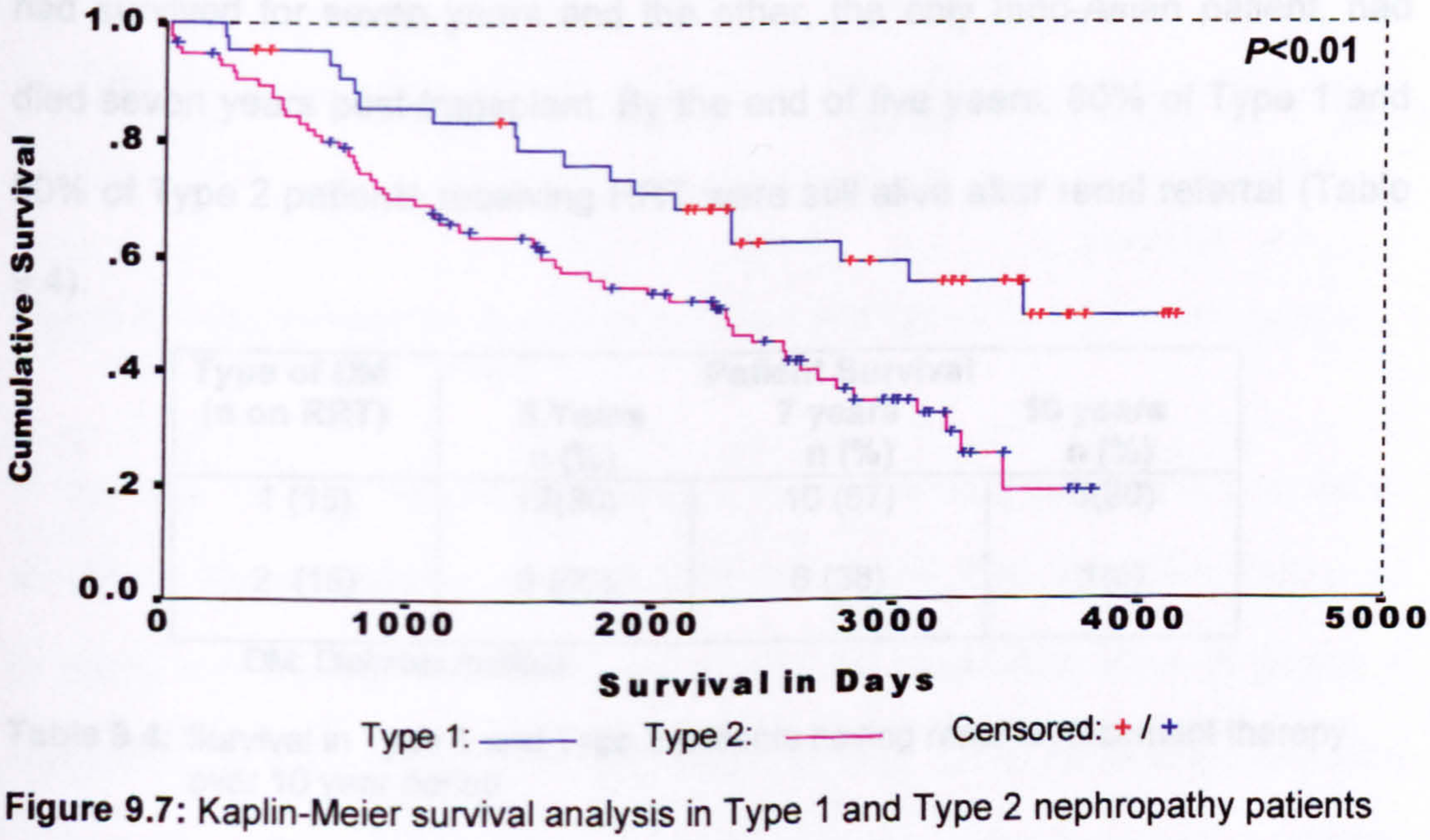

Figure 9.7: Kaplin-Meier survival analysis in Type 1 and Type 2 nephropathy patients

Five years after renal referral 68% of Type 1 and 44% of Type 2 patients were still alive (Table 9.3).

Type of Diabetes	Patients Survival 5 Years n (%)	7 years n (%)	10 years n (%)
1	30 (68)	19 (43)	6 (14)
2	43 (44)	28 (29)	3 (3)

Table 9.3: Patient survival at different time points: main group analysis

Five Type 1 and two Type 2 patients had received renal transplants. Of the Type 1 patients, two had survived for 11 years post-transplant, one for nine years, one for six and one had died after nine years. One of the Type 2 patients had survived for seven years and the other, the only Indo-Asian patient, had died seven years post-transplant. By the end of five years, 80% of Type 1 and 50% of Type 2 patients receiving RRT were still alive after renal referral (Table 9.4).

Type of DM (n on RRT)	Patient Survival 5 Years n (%)	7 years n (%)	10 years n (%)
1 (15)	12(80)	10 (67)	3(20)
2 (16)	8 (50)	6 (38)	1(6)

DM: Diabetes mellitus

Table 9.4: Survival in Type 1 and Type 2 patients having renal replacement therapy over 10 year period

The mean cumulative survival in Type 1 patients on RRT was 3081 (SE±: 316; 95% CI: 2462, 3699) days [8 years] with a median of 3522 (SE±: 506; 95% CI:

2530, 4515) days [10 years] in comparison to a mean of 1934 (SE± 207; 95% CI: 1332, 2535) days [5 years] a median of 2306 (SE±1473; 95% CI: 0,5192) days [6 years] in Type 2 patients receiving RRT. The distribution of survival in Type 1 and Type 2 patients receiving RRT was not the same (χ^2 Logrank: 7.39, df=1, *P*<0.01) (Figure 9.8).

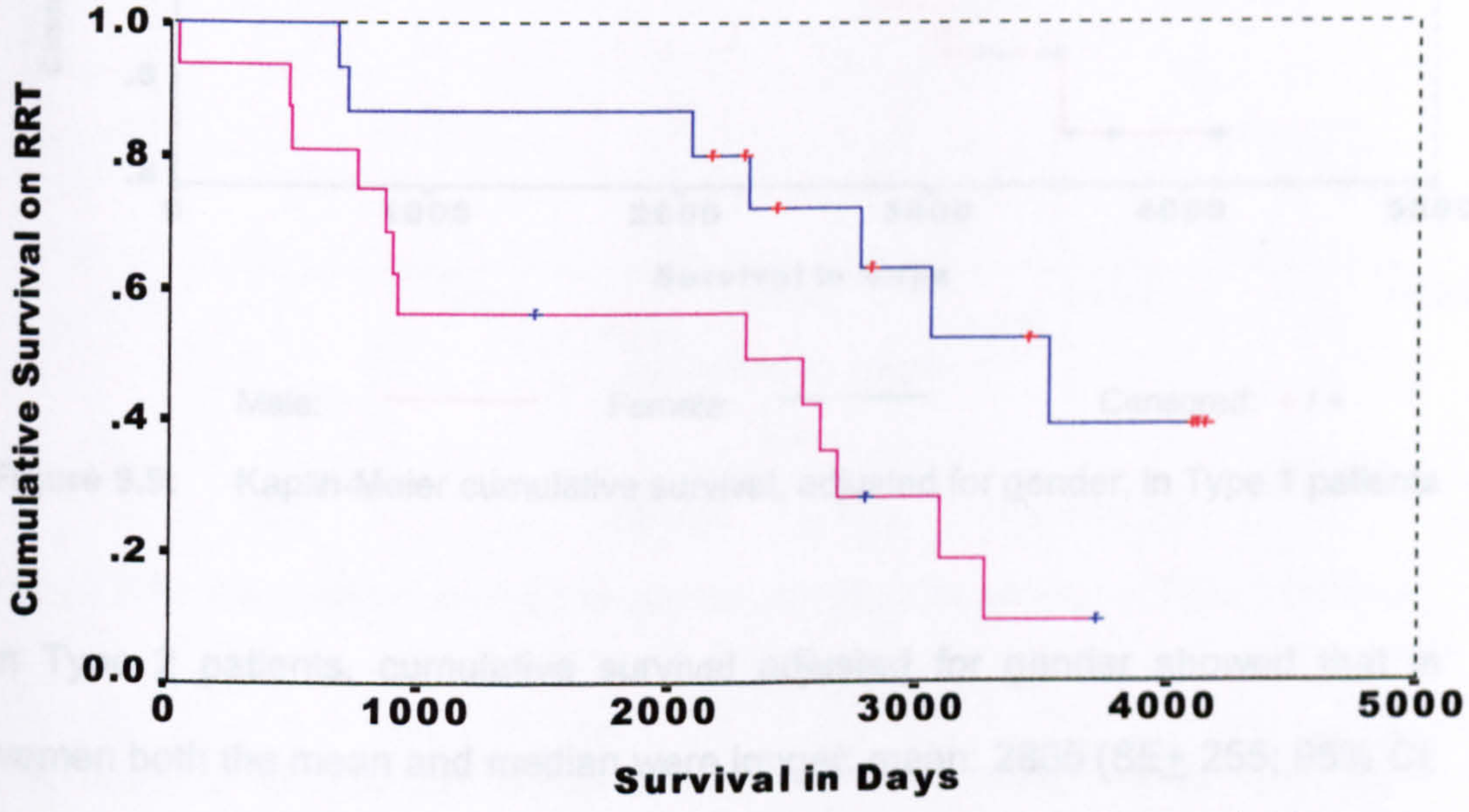

Figure 9.8: Kaplin-Meier survival curve in Type 1 and Type 2 patients receiving renal replacement therapy

In the Type 1 group, adjusted for gender, there was no difference in cumulative survival in women patients, mean of 3073 (SE: ± 348; 95% CI: 2391,3755) days [8 years] and median: 2295 (SE and 95%CI not calculated) days [6 years] in comparison to men; mean: 2124 (SE± 222; 95%CI: 1689, 2560) days [6 years] and median: 2609 (SE±883; 95% CI: 879, 4339) days [7 years] (χ^2 Logrank: 3.07, df=1, *P*=N.S.) (Figure 9.9).

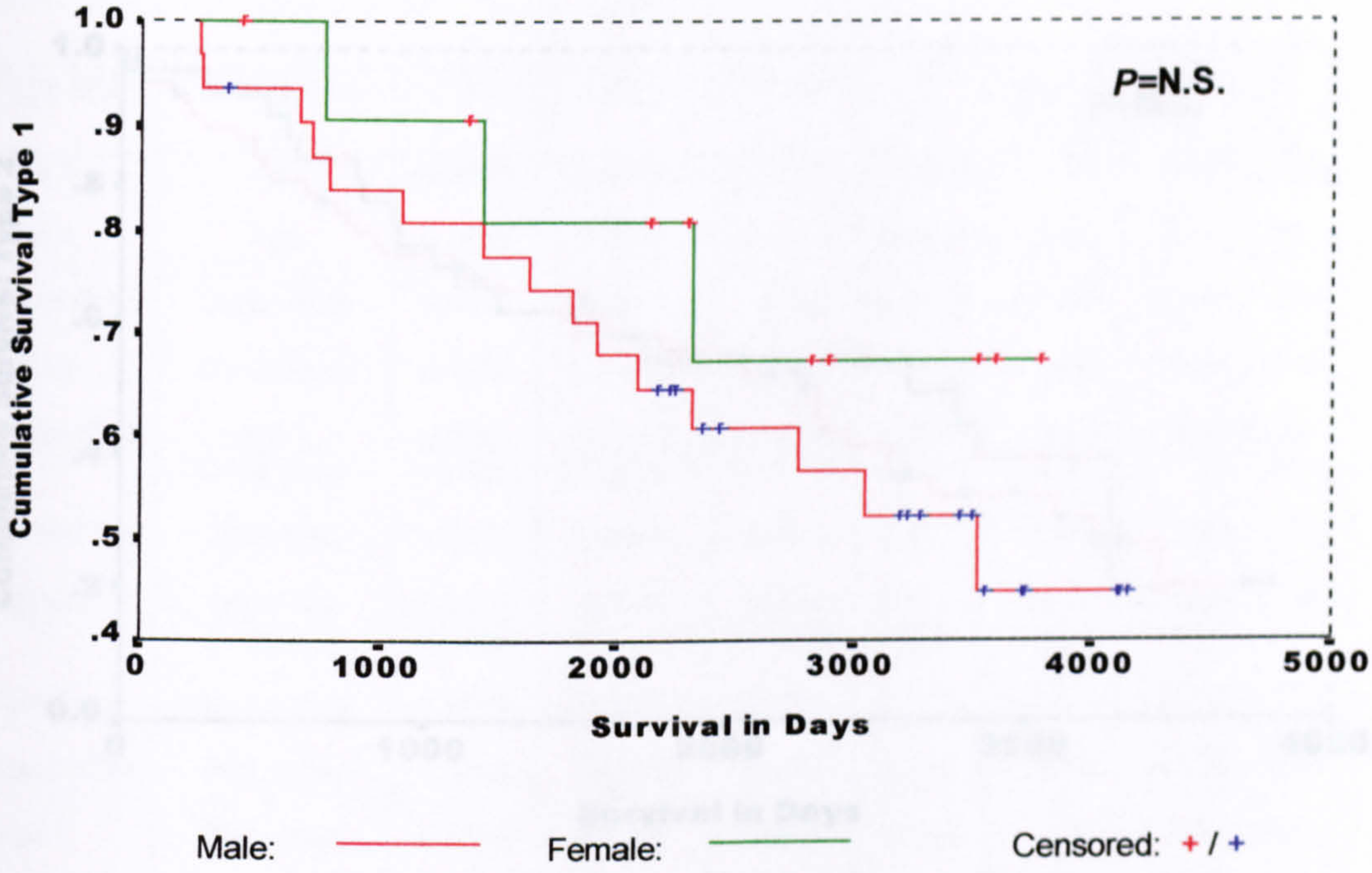

Figure 9.9: Kaplin-Meier cumulative survival, adjusted for gender, in Type 1 patients

In Type 2 patients, cumulative survival adjusted for gender showed that in women both the mean and median were longer: mean: 2865 (SE± 255; 95% CI: 2364, 3366) days [8 years] with a median of 3522 (SE±682; 95% CI: 2185, 4859) days [10 years]) than in men (mean: 2068 (SE±172; 95% CI: 1730, 2406) days [6 years]) with a median of 2247 (SE±282; 95% CI: 1695, 2799) days [6 years] ($\chi^2_{Logrank}$: 0.72, df=1, *P*=N.S.) (Figure 9.10).

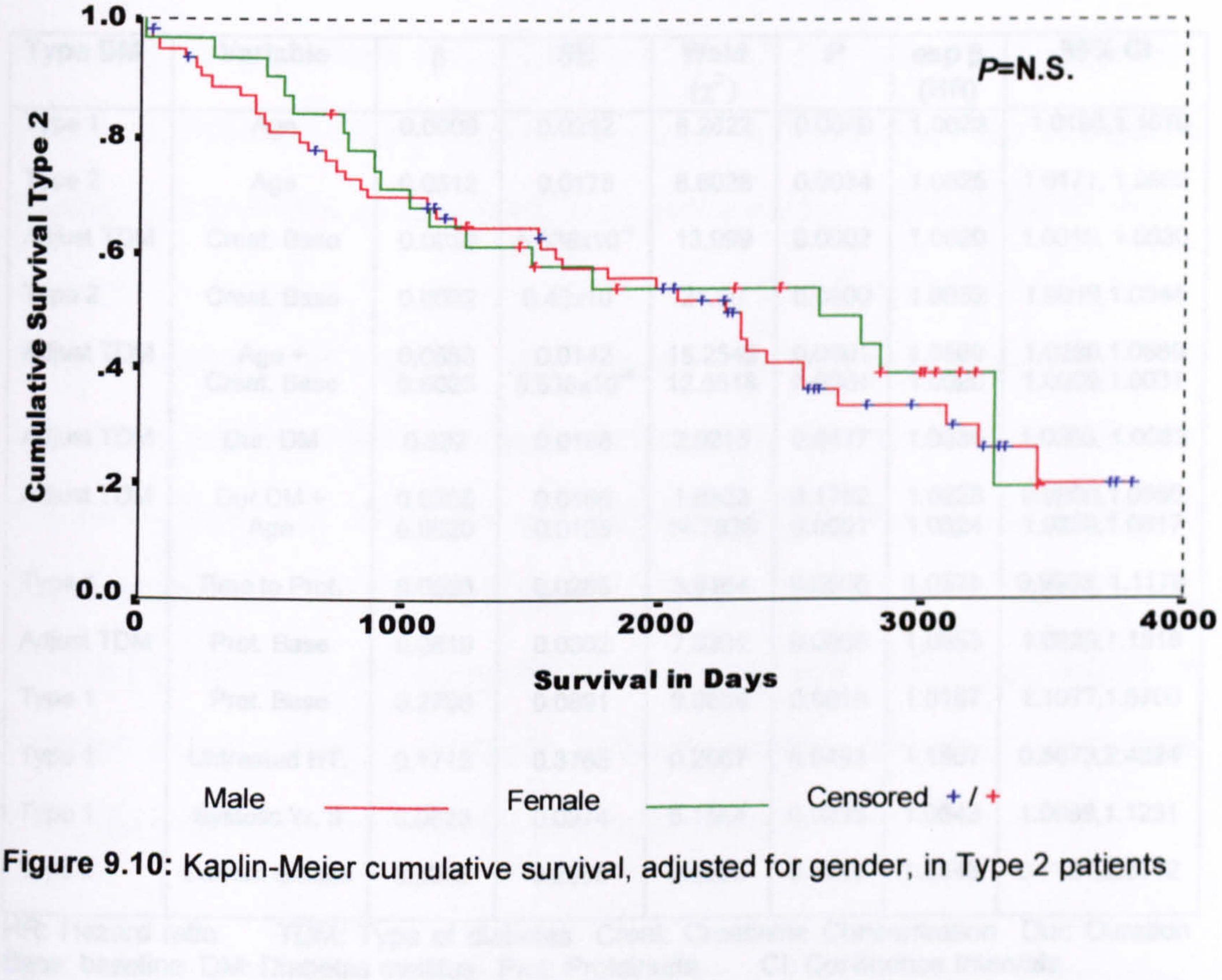

Figure 9.10: Kaplin-Meier cumulative survival, adjusted for gender, in Type 2 patients

The data for all patients indicated that current smoking status was not a significant potential factor affecting survival when adjusted for type of diabetes.

9.5.3 Other Factors Affecting Survival

A number of variables were assessed for significance in terms of survival and hazard using the Cox proportional hazards model in individual groups and when adjusted for type of diabetes. Table 9.5 lists the results for those variables, which were significant, or where the hazard ratio was increased, these will be discussed individually within this results section.

Type DM	Variable	β	SE	Wald (χ^2)	*P*	exp β (HR)	95% CI
Type 1	Age	0.0609	0.0212	8.2822	0.0040	1.0628	1.0196,1.1079
Type 2	Age	0.0512	0.0175	8.6028	0.0034	1.0525	1.0171, 1.0892
Adjust TDM	Creat. Base	0.0020	5.338×10^{-4}	13.999	0.0002	1.0020	1.0010, 1.0030
Type 2	Creat. Base	0.0032	6.43×10^{-4}	24.00	0.0000	1.0032	1.0019,1.0044
Adjust TDM	Age +	0.0553	0.0142	15.2549	0.0001	1.0569	1.0280,1.0869
	Creat. Base	0.0020	5.538×10^{-4}	12.6518	0.0004	1.0020	1.0009,1.0031
Adjust TDM	Dur. DM	0.332	0.0168	3.9215	0.0477	1.0338	1.0003, 1.0683
Adjust TDM	Dur.DM +	0.0255	0.0166	1.8383	0.1752	1.0228	0.9900,1.0566
	Age	0.0520	0.0135	14.7839	0.0001	1.0524	1.0258,1.0817
Type 1	Time to Prot.	0.0556	0.0285	3.8164	0.0508	1.0578	0.9998, 1.1178
Adjust TDM	Prot. Base	0.0819	0.0302	7.3301	0.0068	1.0853	1.0229,1.1516
Type 1	Prot. Base	0.2766	0.0891	9.6659	0.0019	1.3187	1.1077,1.5700
Type 1	Untreated HT.	0.1712	0.3765	0.2067	0.6493	1.1867	0.5673,2.4824
Type 1	Systolic Yr. 3	0.0623	0.0274	5.1564	0.0233	1.0643	1.0086,1.1231
Type 2	Current Smoke	0.2268	0.2868	0.6252	0.4291	1.2546	0.7151,2.2012

HR: Hazard ratio TDM: Type of diabetes Creat: Creatinine Concentration Dur: Duration Base: baseline DM: Diabetes mellitus Prot: Proteinuria CI: Confidence Intervals HT: Hypertension

Table 9.5: Cox proportional hazards model: Assessment of influence of variables on survival in Type 1 and Type 2 nephropathy patients

Cox regression analysis showed that age at referral was a statistically significant factor in cumulative survival in both Type 1 and Type 2 ($P<0.005$ both groups) with the hazard ratio (HR) being similar in both groups (1.06 versus 1.05) (Table 9.5).

When data were adjusted for type of diabetes, duration of diabetes was a statistically significant factor for cumulative survival (HR=1.03, $P<0.05$) (Table

9.5). The addition of age as a covariate, resulted in duration of diabetes becoming non-significant (HR=1.02, *P*=N.S.) but age was highly significant (HR=1.05, *P*<0.001) (Table 9.5). The individual addition of systolic or diastolic pressures at baseline, as covariates, did not change the statistical significance.

Serum creatinine at baseline was another factor that was significant for cumulative survival when data were adjusted for type of diabetes (HR=1.0, *P*<0.001) (Table 9.5). When the groups were analysed separately, it was still significant in Type 2 patients (HR=1.00, *P*<0.001), but not in the Type 1 group (*P*=N.S.) (Table 9.5). Separate addition of systolic and diastolic pressures at baseline as covariates to creatinine levels at baseline, resulted in creatinine being the only significant factor for survival (*P*<0.001).

When adjusted for type of diabetes, proteinuria at baseline was a significant factor for survival (HR=1.09, *P*<0.01), but when the groups were investigated separately, proteinuria at baseline was very significant in Type 1 patients (HR=1.3, *P*<0.005) but not in the Type 2 group (*P* =N.S.) (Table 9.5).

In those patients with hypertension, the absence of anti-hypertensive therapy was not a significant factor on cumulative survival but in Type 1 patients the hazard ratio was increased (HR=1.2) (Table 9.5). In Type 1 patients systolic pressure in year 3 after referral was a significant factor in survival (*P*<0.05) although the HR was only slightly raised (HR=1.06). Current smoking also

increased the hazard ratio (HR=1.25) in Type 2 patients although this was not significant (*P*=N.S.) (Table 9.5).

Hypertension, systolic (except in year 3) and diastolic pressures at baseline and for the seven subsequent years, a history of smoking, glycaemic control (HbA1) at baseline, and for 5 years pre- and 5 years post-baseline, or defaulting from clinic visits were not significant factors in cumulative survival in either group.

9.5.4. *Comparison of Data Between Ethnic Groups*

The maximum survival time in White patients was 11 years in Type 1 patients (n=3) and 10 years in the Type 2 group (n=1) (Figure 9.11). One Indo-Asian and one Black patient survived for 10 years (both Type 2), two Black Type 1 and one Indo-Asian Type 1 patient survived for 9 years. (Figure 9.11).

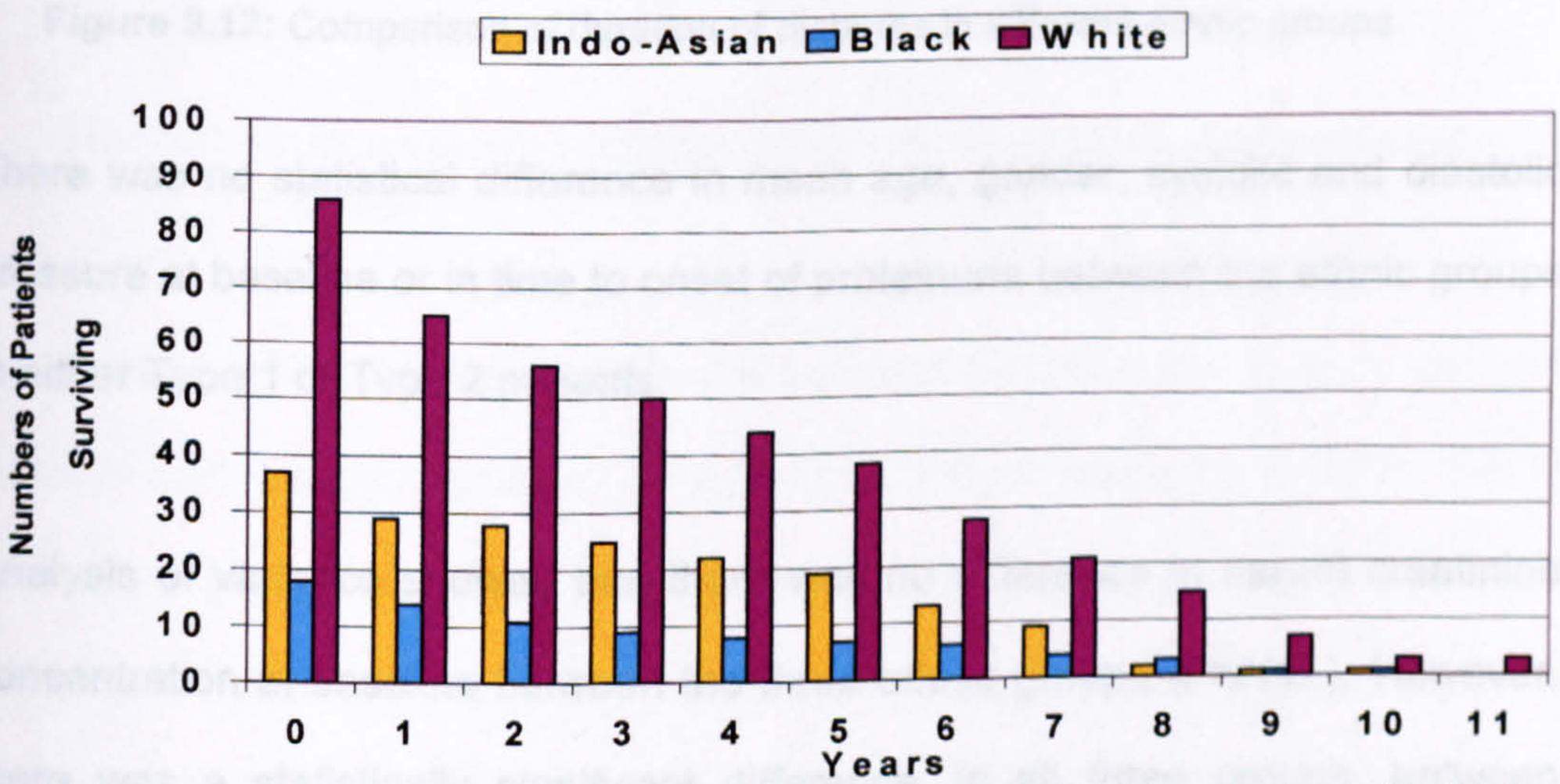

Figure 9.11: Numbers of patients surviving at the end of each year in each ethnic group

Analysis of variance demonstrated that duration of diabetes in Type 1 White patients was significantly longer [mean: 25(SD±8) years] than in Indo-Asian [mean: 17 (SD±4) years] and Black [mean: 18(SD±5) years] Type 1 patients (P<0.05) but there was no difference in Type 2 patients (Figure 9.12)

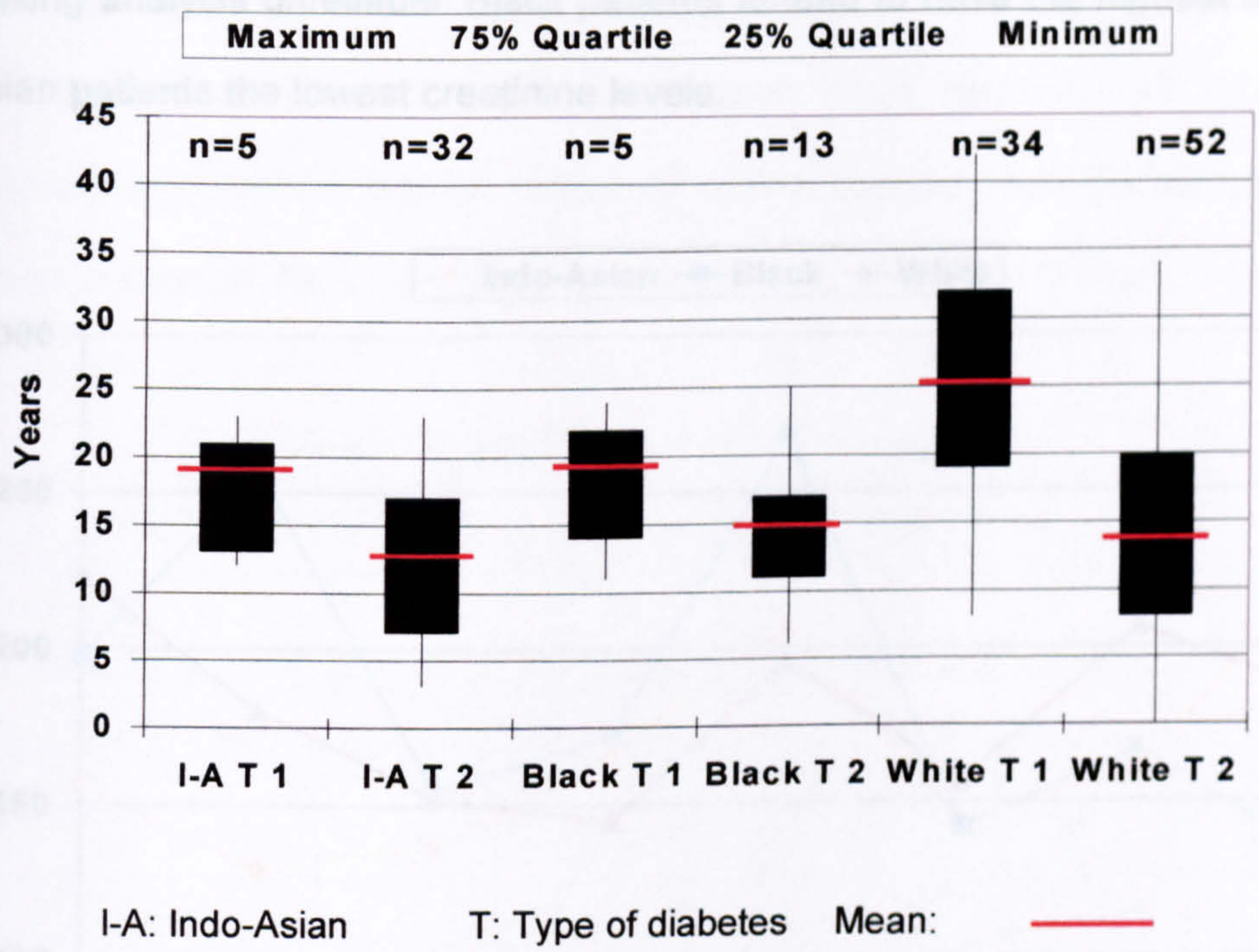

Figure 9.12: Comparison of duration of diabetes in different ethnic groups

There was no statistical difference in mean age, gender, systolic and diastolic pressure at baseline or in time to onset of proteinuria between the ethnic groups in either Type 1 or Type 2 patients.

Analysis of variance showed that there was no difference in serum creatinine concentration at baseline between the three ethnic groups (P=N.S.). However, there was a statistically significant difference, in all three groups, between

creatinine at baseline and year 1 (P<0.001) and when year 2 creatinine was added as a covariate, between baseline and year 2 (*P*<0.05) (Figure 9.13). By year 6 there were no differences from baseline in creatinine levels. By year seven, the number of patients was low in both the Black and Indo-Asian groups, thus making analysis unreliable. Black patients tended to have the highest and Indo-Asian patients the lowest creatinine levels.

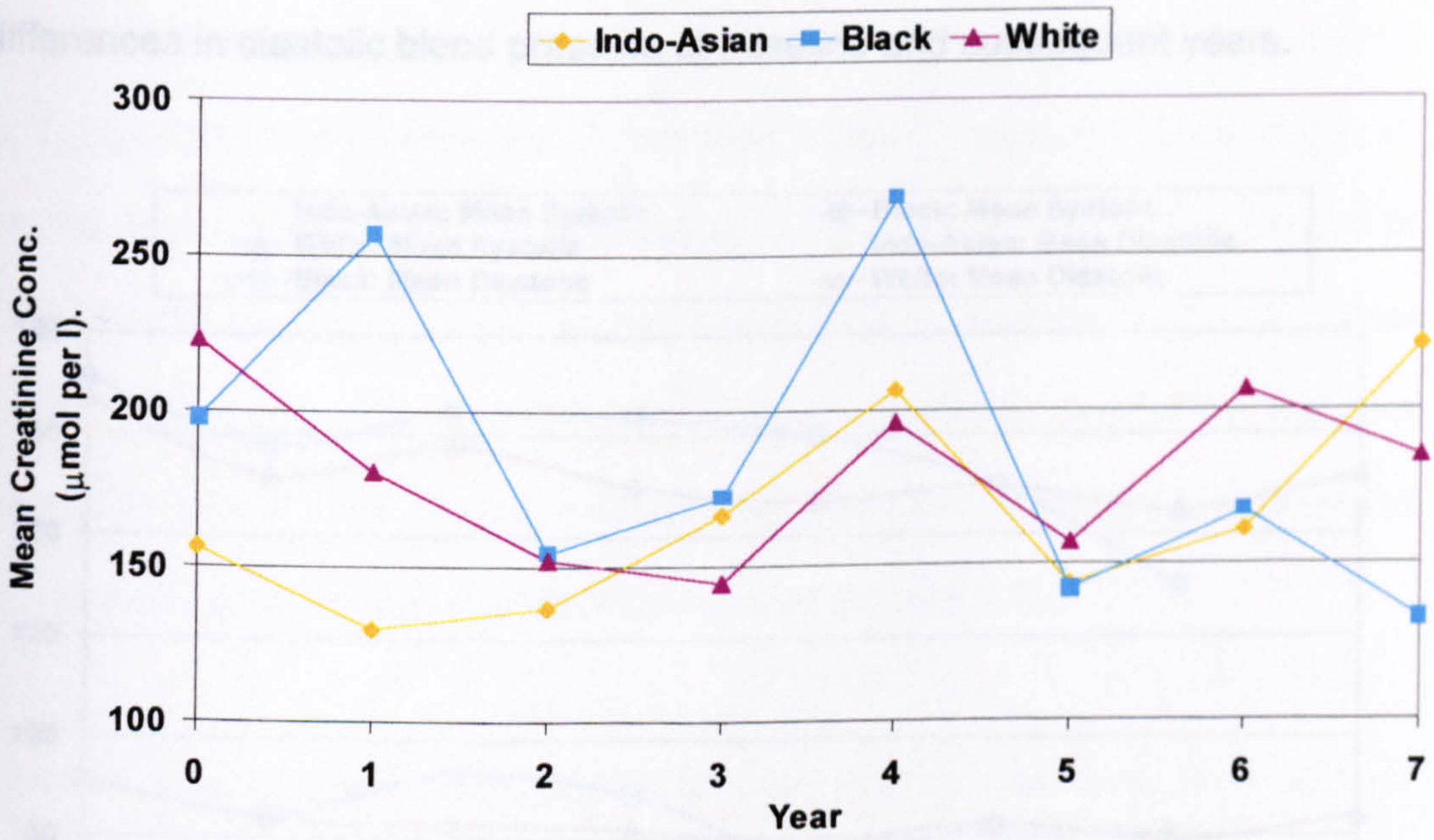

Figure 9.13: Mean serum creatinine levels over seven years: Comparison of ethnic groups

Comparison of systolic blood pressure between baseline and year 1, showed that this significantly decreased (*P*<0.001), and there was no difference due to ethnicity (Figure 9.14). When year 2 was added as a covariate, again there was a significant difference in all three groups (*P*=0.01) as there was when year 3 was added (*P*<0.05). There were no significant differences in systolic pressure

after year 3, possibly due to the small numbers of Indo-Asian and Black patients. There was a statistically significant difference between diastolic pressure at baseline and at year 1 (P=0.001) but not between baseline and year 2, although by year 3 the difference had again become significant (P=0.05) (Figure 9.14). There was no data for Black patients in year seven. None of these differences were due to ethnicity although Black patients had higher systolic pressures over the first four years. After year 3, there were no statistical differences in diastolic blood pressure at baseline and subsequent years.

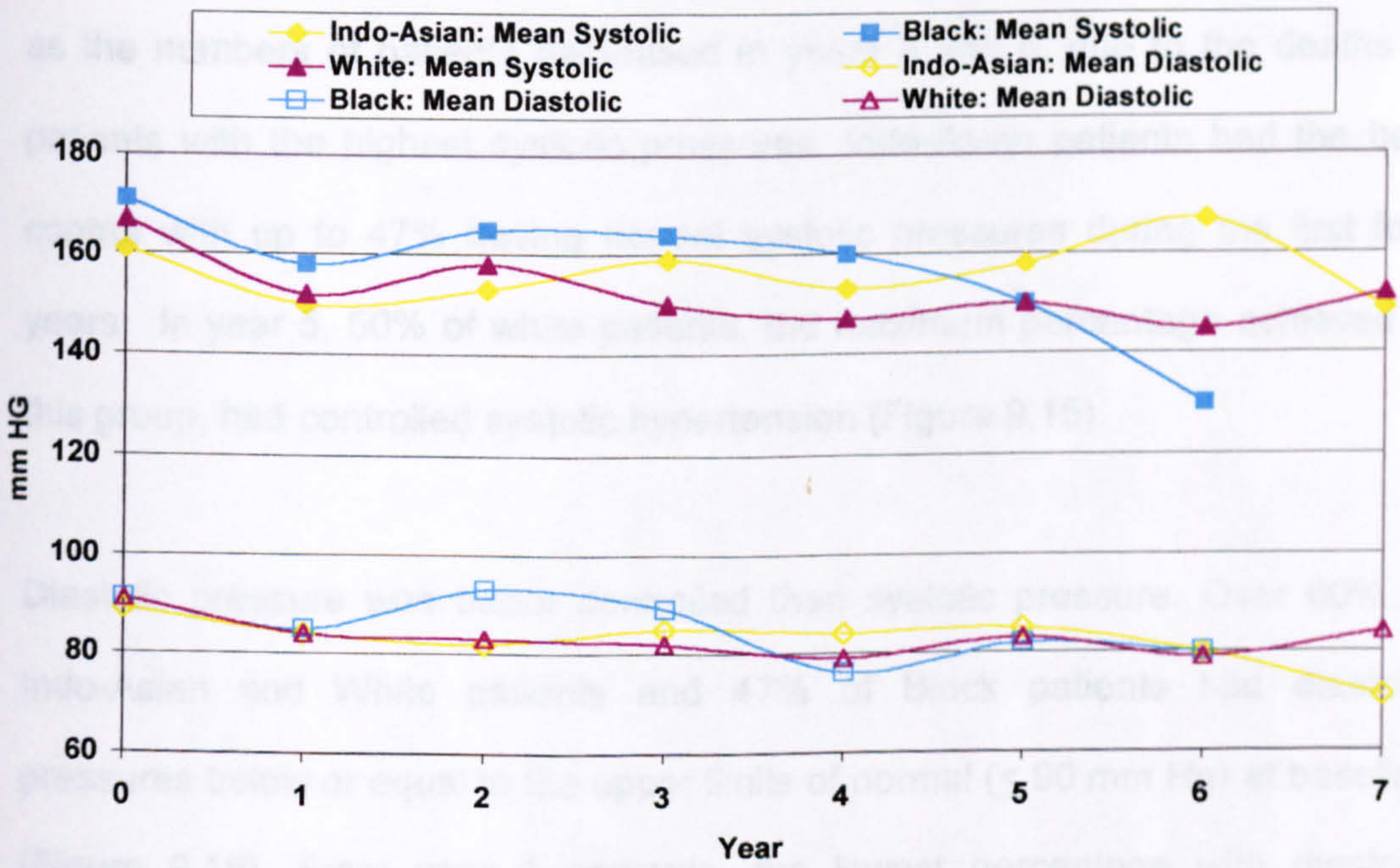

Figure 9.14: Systolic and diastolic blood pressures over seven years: Comparison of ethnic groups

Thirty one (84%) of Indo-Asian, all 18 (100%) Black and 75 (87%) of White patients were hypertensive at baseline (P<0.05) with 13 (42%) Indo-Asian, 8

(44%) Black and 36 (49%) of White hypertensive patients not receiving anti-hypertensive treatment (*P*=N.S.). Of those with treated hypertension, 19 (40%) White, 8 (32%) Indo-Asian and 3 (30%) Black patients were on ACEI while 7 (70%) Black, 13 (52%) Indo-Asian and 18 (38%) White patients required more than one type of anti-hypertensive drug.

At baseline less than 25% of each of the groups had systolic pressures below or equal to the upper limit of normal (≤140 mm Hg) (Figure 9.15). From years 1 to 4, Black patients had the worst controlled systolic pressure but this improved as the numbers of patients decreased in years 5 and 6, due to the deaths of patients with the highest systolic pressures. Indo-Asian patients had the best control with up to 47% having normal systolic pressures during the first four years. In year 5, 50% of white patients, the maximum percentage achieved in this group, had controlled systolic hypertension (Figure 9.15).

Diastolic pressure was better controlled than systolic pressure. Over 60% of Indo-Asian and White patients and 47% of Black patients had diastolic pressures below or equal to the upper limits of normal (≤ 90 mm Hg) at baseline (Figure 9.16). From year 1 onwards, the lowest percentage with diastolic pressures within the normal limits was 60%.

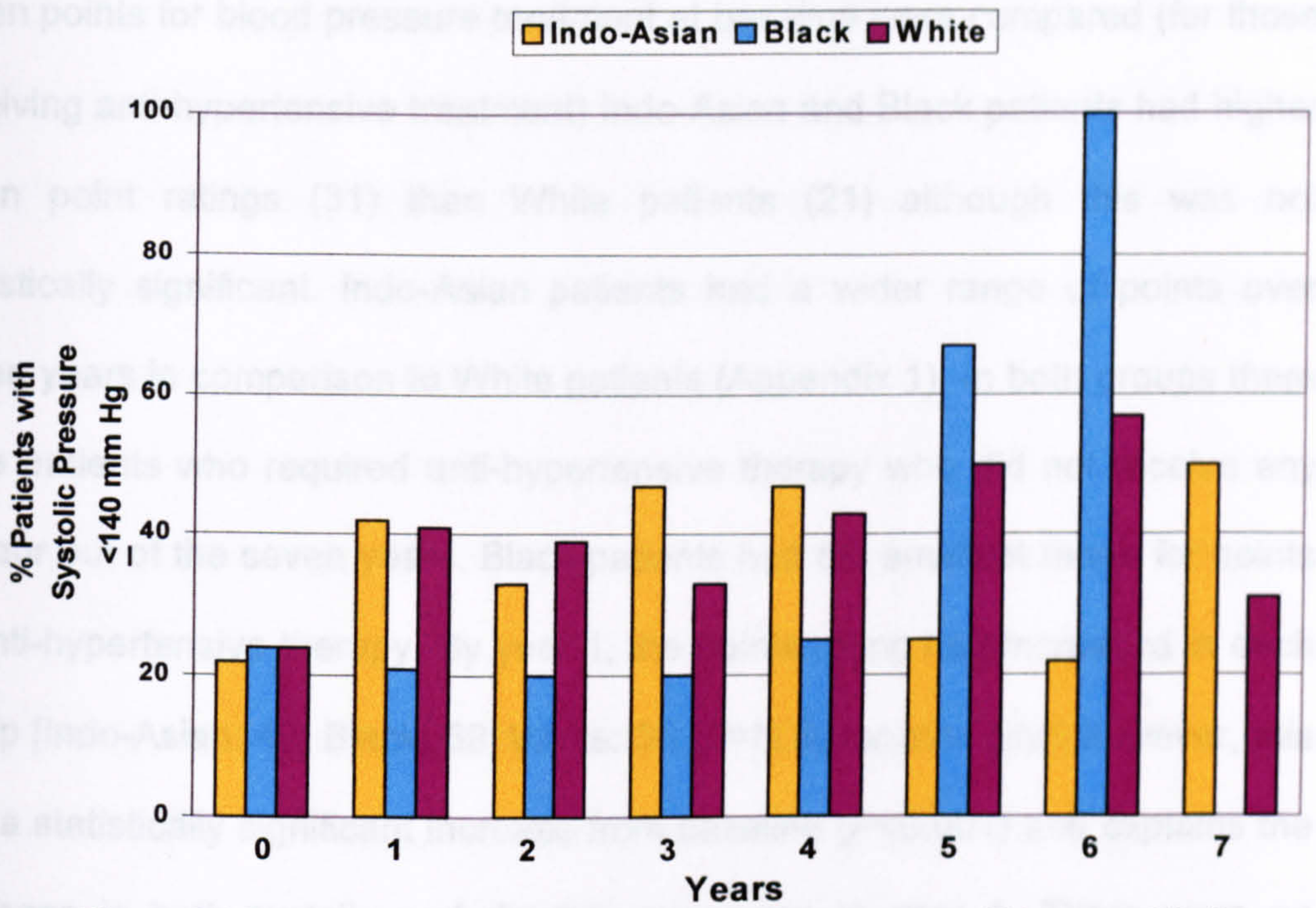

Figure 9.15: Percentage of patients with systolic pressures within normal limits over seven years: Comparison of ethnic groups

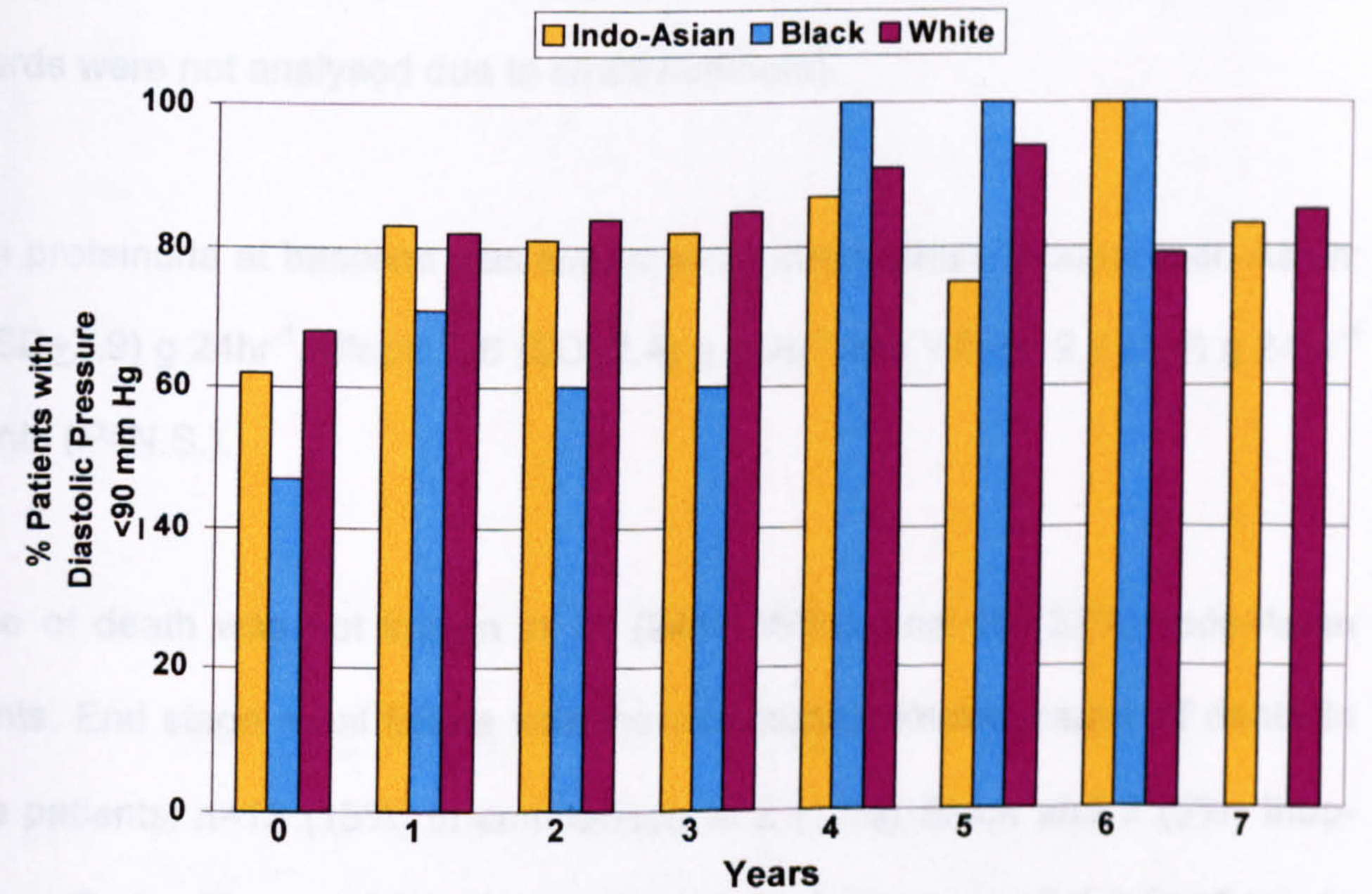

Figure 9.16: Percentage of patients with diastolic pressures within normal limits over seven years; comparison of different ethnic groups

When points for blood pressure treatment at baseline were compared (for those receiving anti-hypertensive treatment) Indo-Asian and Black patients had higher mean point ratings (31) than White patients (21) although this was not statistically significant. Indo-Asian patients had a wider range of points over seven years in comparison to White patients (Appendix 1). In both groups there were patients who required anti-hypertensive therapy who did not receive any for four out of the seven years. Black patients had the smallest range for points of anti-hypertensive therapy. By year 1, the points rating had increased in each group [Indo-Asian: 42; Black: 39; White: 36 (*P*=N.S. for ethnicity)] however, this was a statistically significant increase from baseline (*P*<0.001) and explains the decrease in both systolic and diastolic pressures in year 1. There were no significant differences in points rating from baseline in years 2 and 3 (years 4 onwards were not analysed due to small numbers).

Mean proteinuria at baseline was similar in all three ethnic groups: Indo-Asian: 2.8 (SD$\pm$2.9) g $24hr^{-1}$, Black: 3.6 (SD$\pm$2.4) g $24hr^{-1}$ and White: 2.7 (3.4) g $24hr^{-1}$ patients (*P*=N.S.).

Cause of death was not known in 21 (24%) White and 10 (27%) Indo-Asian patients. End stage renal failure was the commonest known cause of death in White patients: n=13 (15%) in comparison to 2 (11%) Black and 2 (5%) Indo-Asian patients. Three (16%) Black patients died of myocardial infarctions in comparison to 5 (6%) White and 1 (5%) Indo-Asian patients. Three (16%) Black

patients died of congestive cardiac failure in comparison to 2 (2%) White and 2 (5%) Indo-Asian patients. Cerebrovascular accidents were responsible for 2 (11%) deaths in Black patients, 3 (3%) in White patients and 2 (5%) Indo-Asian deaths. Statistical analysis was not performed due to the large number of deaths from unknown cause.

9.5.5 *Comparison of Survival in Different Ethnic Groups*

There was no statistical difference in cumulative survival in Indo-Asian Type 1 and Type 2 patients (χ^2 Logrank: 0.18, df=1, *P*=N.S.) (Figure 9.17).

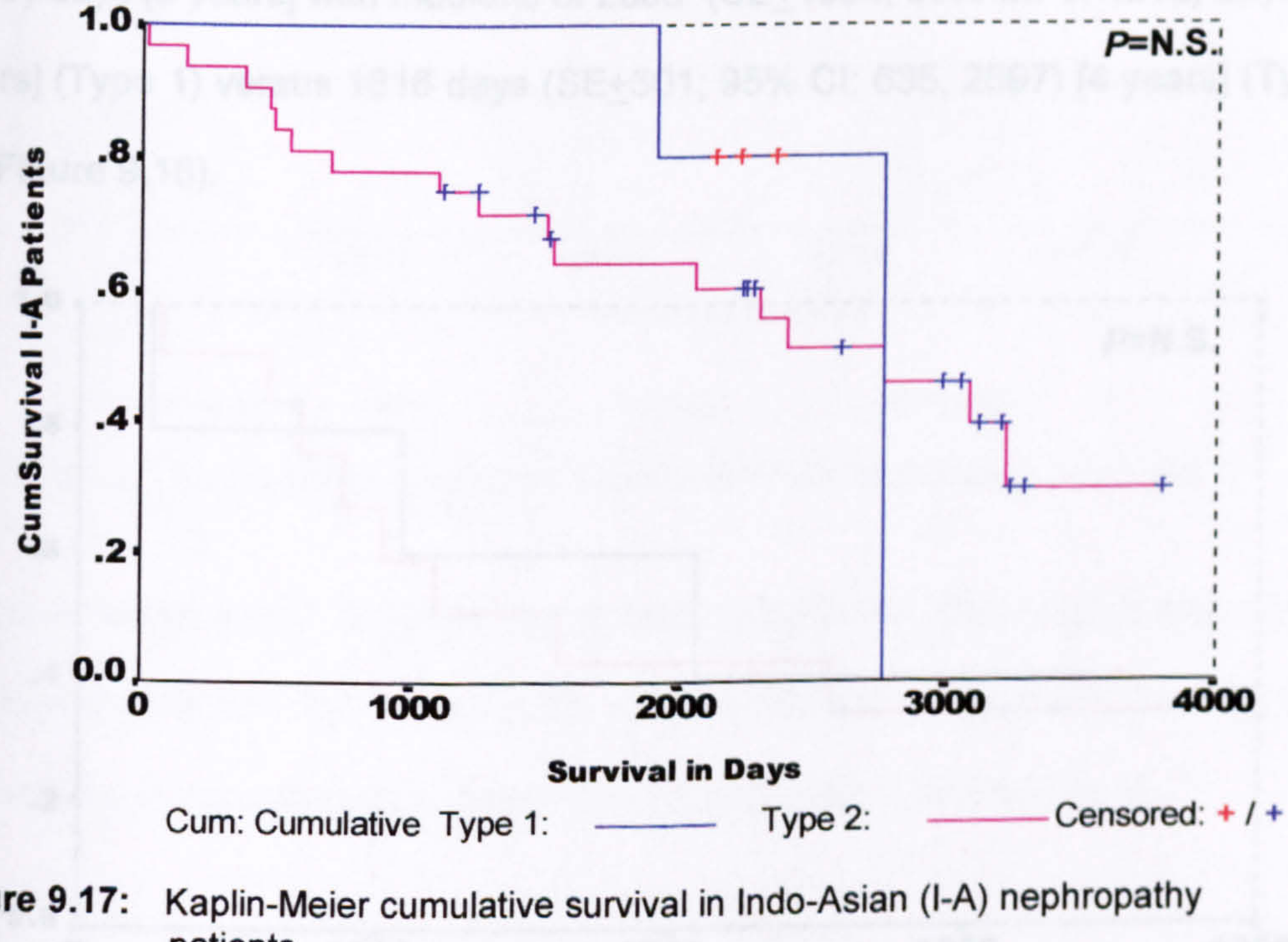

Figure 9.17: Kaplin-Meier cumulative survival in Indo-Asian (I-A) nephropathy patients

The mean for the Type 1 Indo-Asian group was 2598 (SE: 215; 95% CI: 2178, 3019) days [7 years] in comparison to Type 2 patients; mean: 2360 (SE: 246; 95% CI: 1878, 2842) days [6 years] with medians of 2768 (SE±0 [n=5, 2 deaths and 3 censored]) days [7years] (Type 1) versus 2765 days (SE±489; 95% CI: 1825, 3705) [7 years] (Type 2).

Similarly, there was no statistical difference between the groups in cumulative survival in Black patients (χ^2 Logrank: 0.02, df=1, *P*=N.S.) (Figure 9.18). The mean for Type 1 patients was 2093 (SE: ± 582; 95% CI: 953, 3233) days [6 years] in comparison to the mean for the Type 2 group of 2107 (SE± 387; 95% CI: 1260, 2775) days [6 years] with medians of 2086 (SE±1084; 95% CI: 0, 4212) days [6 years] (Type 1) versus 1616 days (SE±501; 95% CI: 635, 2597) [4 years] (Type 2) (Figure 9.18).

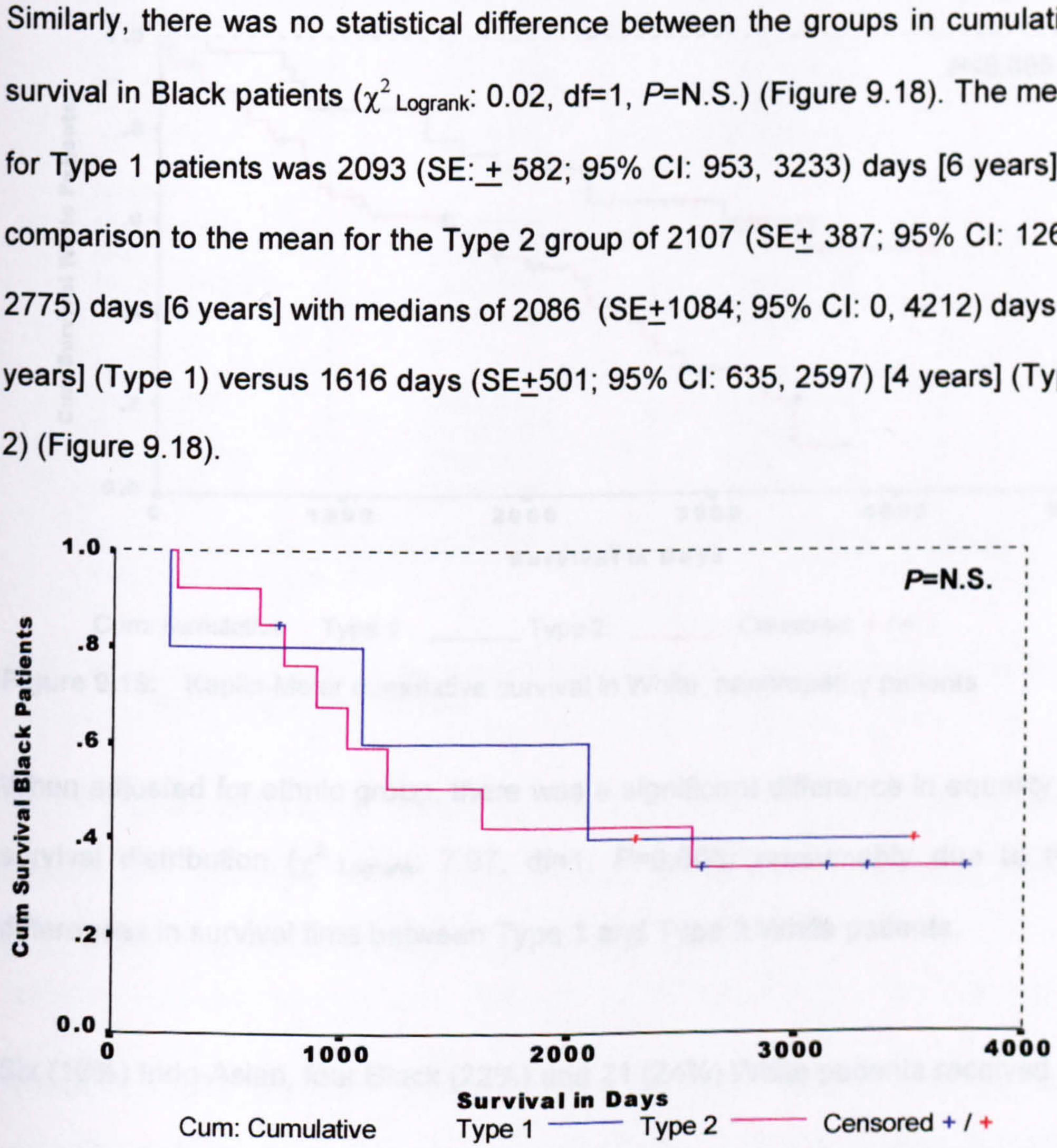

Figure 9.18: Kaplin-Meier cumulative survival in Black nephropathy patients

However, the cumulative survival in Type 1 White patients was significantly longer; mean: 3050 (SE± 248; 25% CI: 2565,3535) days [8 years] than in the Type 2 group; mean: 1950 (SE± 184; 95% CI: 1589, 2310) days [5 years] with medians of 2240 days [6 years] (Type 1) and 2247 (SE±416; 95% CI: 1431, 3063) days [6 years] (Type 2) ($\chi^2_{Logrank}$: 9.56, df=1, P<0.005). (Figure 9.19).

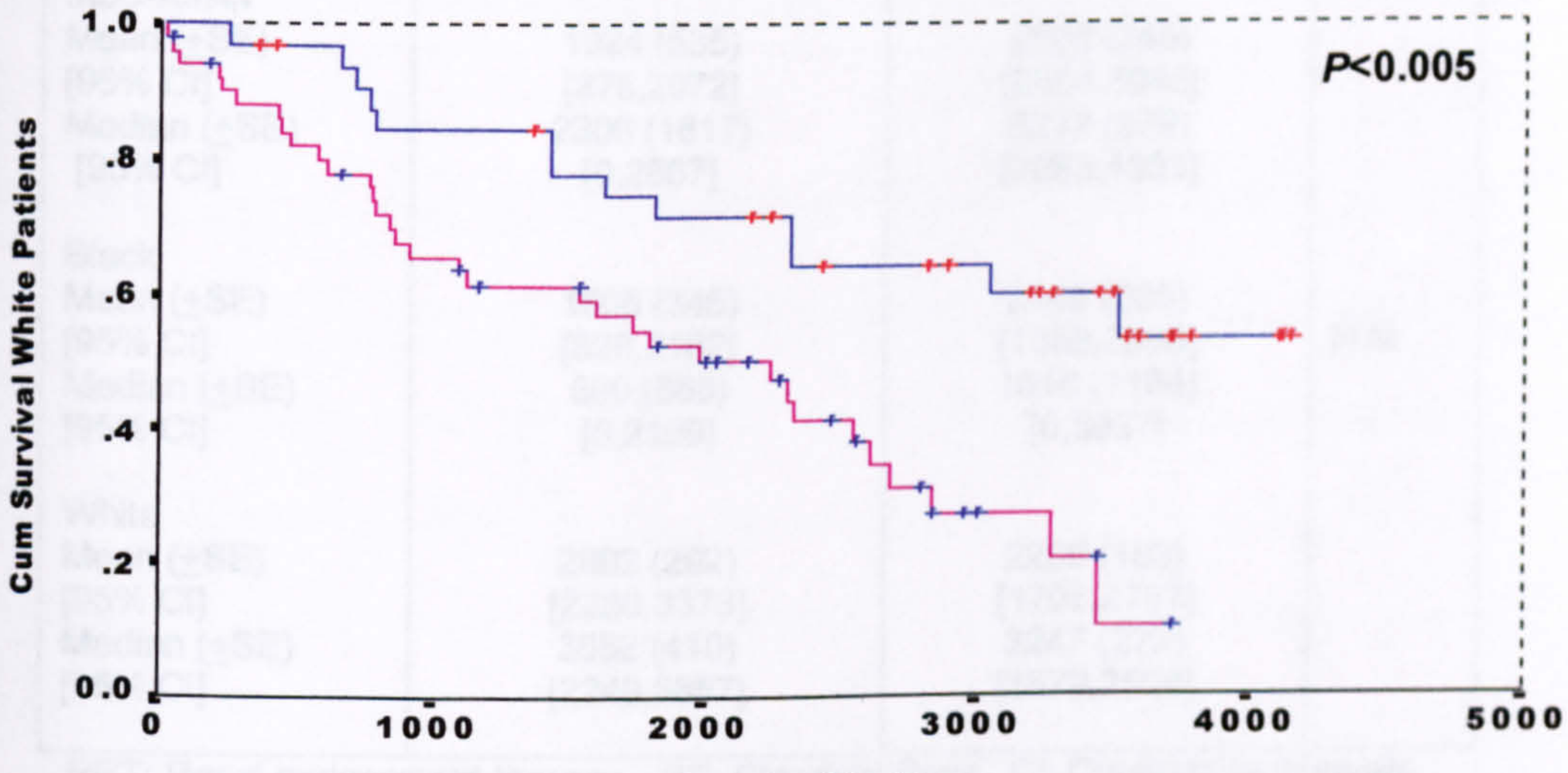

Figure 9.19: Kaplin-Meier cumulative survival in White nephropathy patients

When adjusted for ethnic group, there was a significant difference in equality of survival distribution ($\chi^2_{Logrank}$: 7.97, df=1, P=0.005) presumably due to the differences in survival time between Type 1 and Type 2 White patients.

Six (16%) Indo-Asian, four Black (22%) and 21 (24%) White patients received

RRT (*P*=N.S.). There was no statistical difference in equality of survival distributions when adjusted for RRT and ethnic group (χ^2 Logrank: 0.22, df=1, *P*=N.S.) or when compared with patients who did not receive RRT (χ^2Logrank: 0.30, df=1, *P*=N.S.) (Table 9.6).

Ethnic Group	Cumulative Survival On RRT (days)	Cumulative Survival Without RRT (days)	*P*
INDO-ASIAN			
Mean (±SE)	1924 (535)	2565 (245)	
[95% CI]	[875,2972]	[2084,3046]	
Median (±SE)	2306 (1817)	3217 (579)	
[95% CI]	[0,2867]	[2083,4351]	
Black			
Mean (±SE)	1505 (345)	2142 (385)	
[95% CI]	[828,2182]	[1388,2896]	N.S.
Median (±SE)	890 (668)	1616 (1184)	
[95% CI]	[0,2199]	[0,3937]	
White			
Mean (±SE)	2802 (292)	2236 (183)	
[95% CI]	[2230,3373]	[1707,2787]	
Median (±SE)	3052 (410)	2247 (272)	
[95% CI]	[2249,3857]	[1879,2594]	

RRT: Renal replacement therapy SE: Standard Error CI: Confidence Intervals
N.S.: Not significant

Table 9.6: Cumulative survival for different ethnic groups dependent on treatment with renal replacement therapy

After five years of follow up, 67% of Indo-Asian, 25% of Black and 76% of White patients were still alive. By ten years of follow up, 17% of Indo-Asians, 25% of Black and 43% of White patients were still alive.

In Black patients who were current smokers the mean survival time was 889 (SE±269; 95% CI: 362, 1417) days [2 years] with a median of 1096 (SE±891;

95% CI: 0,2842) days [3 years] in comparison to non-current smokers where mean survival time was 2529 (SE± 376; 95% CI: 1791, 3267) days [7 years] with a median of 2550 days [7 years]. The equality of survival distribution was found not to be equal ($\chi^2_{Logrank}$: 5.99, df=1, P<0.05) (Figure 9.20). There was no difference in survival time between current smokers and non-smokers in the other two ethnic groups.

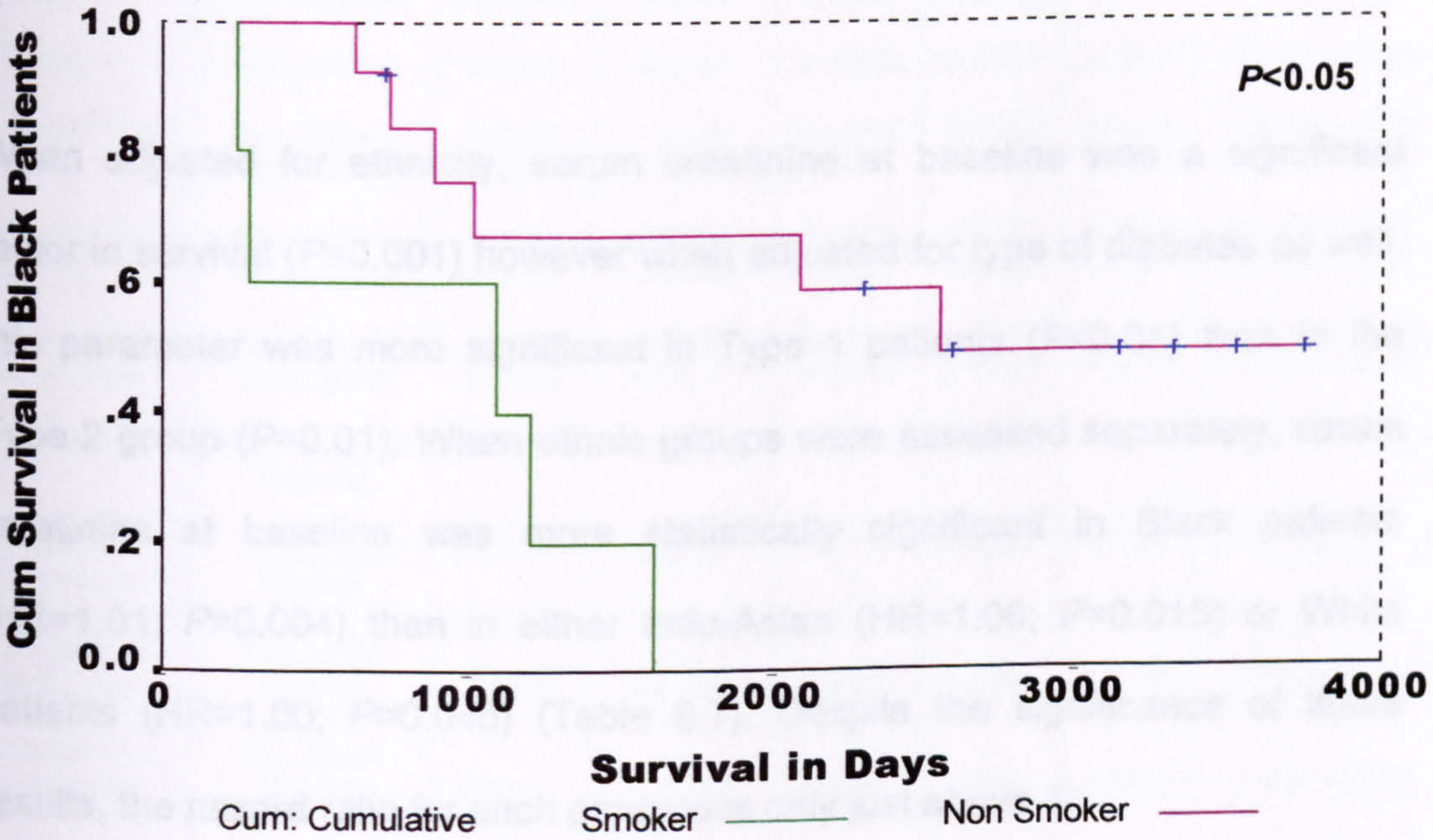

Figure 9.20: Kaplin-Meier cumulative survival in Black patients due to current smoking habits

Cox regression analysis showed that Black patients who were current smokers had an increased hazard ratio (HR) of 4.57 (P=0.25) (Table 9.7). A history of smoking was also a significant factor in reducing survival in black patients (HR=4.62, P=0.017) (Table 9.7).

9.5.6 Other Factors Affecting Survival

Several variables were assessed for significance in terms of survival and hazard using the Cox proportional hazard model in individual groups, when adjusted for ethnicity and when adjusted for type of diabetes. Table 9.7 lists the results for those variables which were significant or where the hazard ratio was increased, and these will be discussed individually within this results section.

When adjusted for ethnicity, serum creatinine at baseline was a significant factor in survival (P=0.001) however when adjusted for type of diabetes as well, this parameter was more significant in Type 1 patients (P<0.01) than in the Type 2 group (P=0.01). When ethnic groups were assessed separately, serum creatinine at baseline was more statistically significant in Black patients (HR=1.01; P=0.004) than in either Indo-Asian (HR=1.00; P=0.015) or White patients (HR=1.00; P=0.046) (Table 9.7). Despite the significance of these results, the hazard ratio for each group was only just above 1.

Ethnic Group	Variable	β	SE	Wald (χ^2)	*P*	exp β (HR)	95% CI
ADJUST E	Creat. Base	0.0017	$5.12x10^{-4}$	10.3826	0.0013	1.0017	1.0006,1.0027
WHITE	Creat. Base	0.0012	$5.88x10^{-4}$	3.9958	0.0456	1.0012	1.0000, 1.0023
BLACK	Creat. Base	0.0075	0.0026	8.29	0.004	1.0075	1.0024, 1.0126
INDO-ASIAN	Creat. Base	0.0035	0.0015	5.8753	0.0154	1.0035	1.0007, 1.0064
WHITE	Age Referral	0.0627	0.0143	19.2289	0.0000	1.0647	1.0353, 1.0950
ADJUST E TYPE 1	Age Referral	0.0471	0.0176	7.1956	0.0073	1.0483	1.0128, 1.0850
ADJUST E TYPE 2	Age Referral	0.0541	0.0211	6.5753	0.0108	1.0556	1.0128, 1.1001
WHITE	Age Referral	0.0627	0.0143	19.2289	0.0000	1.0647	1.0353, 1.0950
ADJUST TDM WHITE	Age Refer + Creat Base	0.0640 0.0018	0.0186 $6.726x10^{-4}$	11.7941 6.8547	0.0006 0.0088	1.0001 1.0018	1.0279, 1.1058 1.0004, 1.0031
ADJUST TDM BLACK	Age Refer + Creat Base	0.0331 0.0091	0.0311 0.0035	1.1313 6.5924	0.2875 0.0102	1.0337 1.0091	0.9725, 1.0987 1.0022, 1.0162
ADJUST TDM INDO-ASIAN	Age Refer + Creat Base	0.0204 0.0029	0.0356 0.0015	0.3285 3.6165	0.5666 0.0572	1.0206 0.0572	0.9518, 1.0944 0.9999, 1.0060
INDO-ASIAN	Time to Renal Referral	0.2238	0.9260	5.843	0.0156	1.2508	1.0432, 1.4997
ADJUST E (INDO-ASIAN)	Not Speak English	0.5433 *	0.2460 *	4.8770 *	0.0272 *	1.7217 *	1.0630,2.7884 *
ADJUST TDM INDO-ASIAN	Not Speak English	0.5573	0.2604	4.5823	0.0323	1.7460	1.0482, 2.9084
ADJUST SEX INDO-ASIAN	Not Speak English	0.8324	0.3158	6.9491	0.0084	2.2989	1.2380, 4.2688
MALE INDO-ASIAN	Not Speak English	0.9676	0.3468	7.7826	0.0053	2.6316	1.3335, 5.1934
FEMALE INDO-ASIAN	Not Speak English	0.3595	0.5484	0.4297	0.5121	1.4326	0.4890, 4.1967
INDO-ASIAN	Prot. Base	0.2857	0.0997	8.2114	0.0042	1.3307	1.0945, 1.6180
BLACK	Prot. Base	0.1752	0.0882	3.9441	0.0470	1.1915	1.0023, 1.4165
BLACK	Current Smoke	1.5194	0.6793	5.0028	0.0253	4.5697	1.2068, 17.303
BLACK	Smoke History	1.5323	0.6421	5.6954	0.0170	4.6290	1.3151, 16.294

HR: Hazard Ratio E: Ethnicity TDM: Type of diabetes Creat: Serum creatinine concentration
Base: Baseline Refer: Referral Prot: Proteinuria CI: Confidence intervals

Table 9.7: Cox proportional hazards model assessing influence of variables on survival in ethnic groups.

Age at referral was also a statistically significant factor for survival in both groups (P<0.01) when adjusted for ethnicity, (HR=1.05 in both groups). In White patients age at referral was very significant (P<0.001, HR=1.06). Age and creatinine at baseline together, adjusted for type of diabetes, were significant covariates in White patients (age: P<0.001; creatinine: P<0.01; although the HR was very close to 1.00) unlike Black patients (age: HR=1.03, P=N.S.; creatinine: HR=1.01, P<0.01,) and Indo-Asian patients (age: HR=1.02, P=N.S.; creatinine: HR=1.00, P=N.S.).

In Indo-Asian patients, time between onset of proteinuria and nephrological referral was a significant factor in cumulative survival (HR=1.25, P<0.05,) unlike White and Black patients, although the median time to referral in all three groups was 1 year. The inability to speak English was also a significant hazard for survival in Indo-Asian patients (HR=1.7, P<0.05). When gender was added as a covariate to inability to speak English in Indo-Asian patients, male patients had an increased HR=2.63 (P=0.005) in comparison to women (HR=1.43, P=N.S.)

When adjusted for ethnic group, proteinuria level at baseline was a statistically significant factor for survival (P<0.005) posing a greater hazard in Indo-Asian (HR=1.33, P<0.005) and Black patients (HR=1.19, P<0.05) than in White patients (P=N.S.) (Table 9.7)

When adjusted for ethnicity and when systolic pressure at baseline was added as a covariate to proteinuria at baseline, the statistical significance of proteinuria decreased ($P<0.05$) while systolic pressure had no significant effect on survival. Removal of systolic pressure as a covariate and addition of diastolic pressure at baseline maintained the significance of proteinuria at baseline ($P<0.005$) but diastolic pressure was not significant. The addition of both systolic and diastolic pressure as covariates resulted in the significance of proteinuria decreasing ($P=0.01$). In the individual ethnic groups, both systolic and diastolic pressures at baseline (added as separate covariates) decreased the significance of proteinuria in Indo-Asian patients (systolic: $P<0.05$; diastolic: $P<0.01$).

Defaulting from routine clinic visits, systolic and diastolic pressures at baseline up to year 3 after referral or HbA1 results either at baseline, for 5 years pre, or 5 years post-baseline examination were not significant as factors in survival.

9.5.7 *Subgroup Analysis of Type 1 Versus Type 2 Patients*

To remove any potential bias due to the presence of advanced renal disease at referral, a further analysis was performed. From the original 141 patients included in the survival analysis, all patients with serum creatinine levels above the upper limit of the normal range (120 μmol l^{-1}) and those who had received RRT were excluded leaving 22 Type 1 and 42 Type 2 patients. The

demographic data were still very similar to the original cohort of patients described in Table 9.1, except that the percentage of White Type 2 patients had decreased from 54% to 45% in the subgroup (Table 9.8).

Demography	Type 1 n (%)	Type 2 n (%)	*P*
Number	22	42	
Male	15 (68)	26 (62)	N.S.
Female	7 (32)	16 (38)	
Indo-Asian	3 (14)	19 (45)	
Black	2 (9)	4 (10)	<0.05
White	17 (77)	19 (45)	
Duration of DM (years)			
Range	8-39	1-26	
Mean (±SD)	22 (8)	13 (7)	<0.001
Median	20	14	
Age (years)			
Range	21-73	39-83	
Mean (±SD)	43 (14)	60 (9)	<0.001
Median	42	60	

N.S.: not significant SD: Standard deviation DM: Diabetes mellitus

Table 9.8: Demographic data for subgroup analysis Type 1 versus Type 2 nephropathy patients

As expected from results in previous chapters, duration of diabetes at onset of proteinuria was shorter in the Type 2 group [mean: 12 (SD±7) years versus Type 1: 20 (SD±9) years] (*P*<0.001). However, there was no statistical difference between the groups in the time to nephrological referral from the diabetic clinic; Type 1 mean: 2 (SD±2) years versus Type 2 mean: 1(SD±1) year (*P*=N.S.).

The groups were well matched for creatinine concentrations at referral; the range in both groups was 65-120 μ mol l^{-1} with a mean in Type 1 patients of 93 (SD$\pm$15) μ mol l^{-1} and median of 92 μ mol l^{-1} in comparison to the Type 2 group: mean: 98 (SD$\pm$ 14) μ mol l^{-1} and median of 98 μ mol l^{-1}.

Twelve (55%) Type 1 and 15 (36%) Type 2 patients had defaulted from routine diabetic clinic visits (*P*=N.S.). A smaller proportion of Type 2 patients were current smokers (Type 2: n=14, 33% versus Type 1: n=9, 41%) (*P*=N.S.) and 55% of both groups had never smoked.

Three (14%) of the Type 1 group and 1 (2%) of the Type 2 patients had survived for 10 years after referral (Figure 9.21).

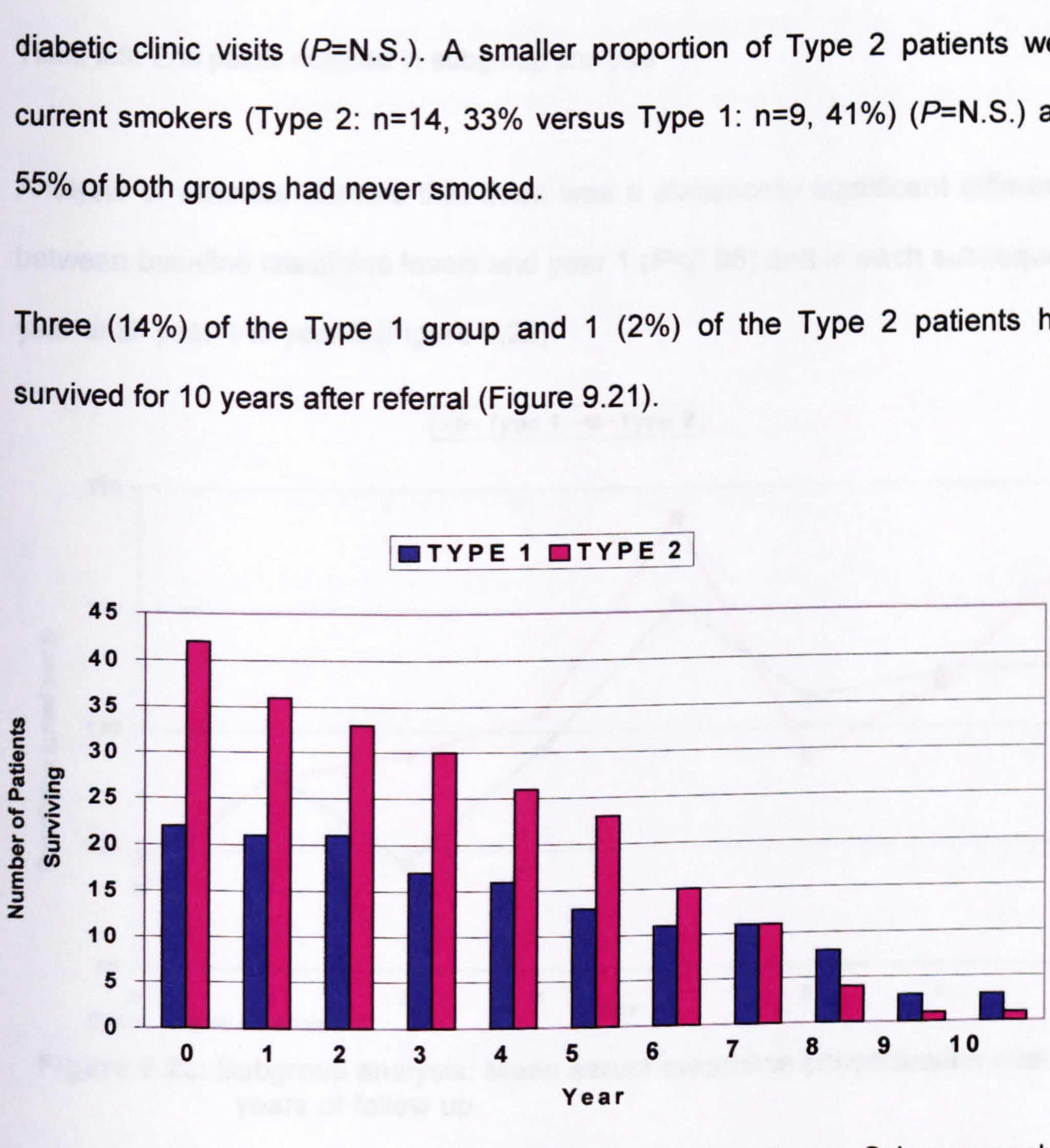

Figure 9.21: Numbers of patients alive at follow up in each year: Subgroup analysis

At the end of the twelve year follow up period, 13 (59%) of Type 1 and 16 (38%) of Type 2 patients were still alive (Table 9.9) but each patient had been followed up for different lengths of time.

End Point	Type 1 n (%)	Type 2 n (%)	*P*
Alive	13 (59)	16 (38)	N.S.
Dead	7 (32)	18 (43)	
Lost to Follow up	2 (9)	8 (19)	

Table 9.9: End points reached in subgroup analysis

Analysis of variance showed that there was a statistically significant difference between baseline creatinine levels and year 1 ($P<0.05$) and in each subsequent year until year 4 to year 5 (Figure 9.22).

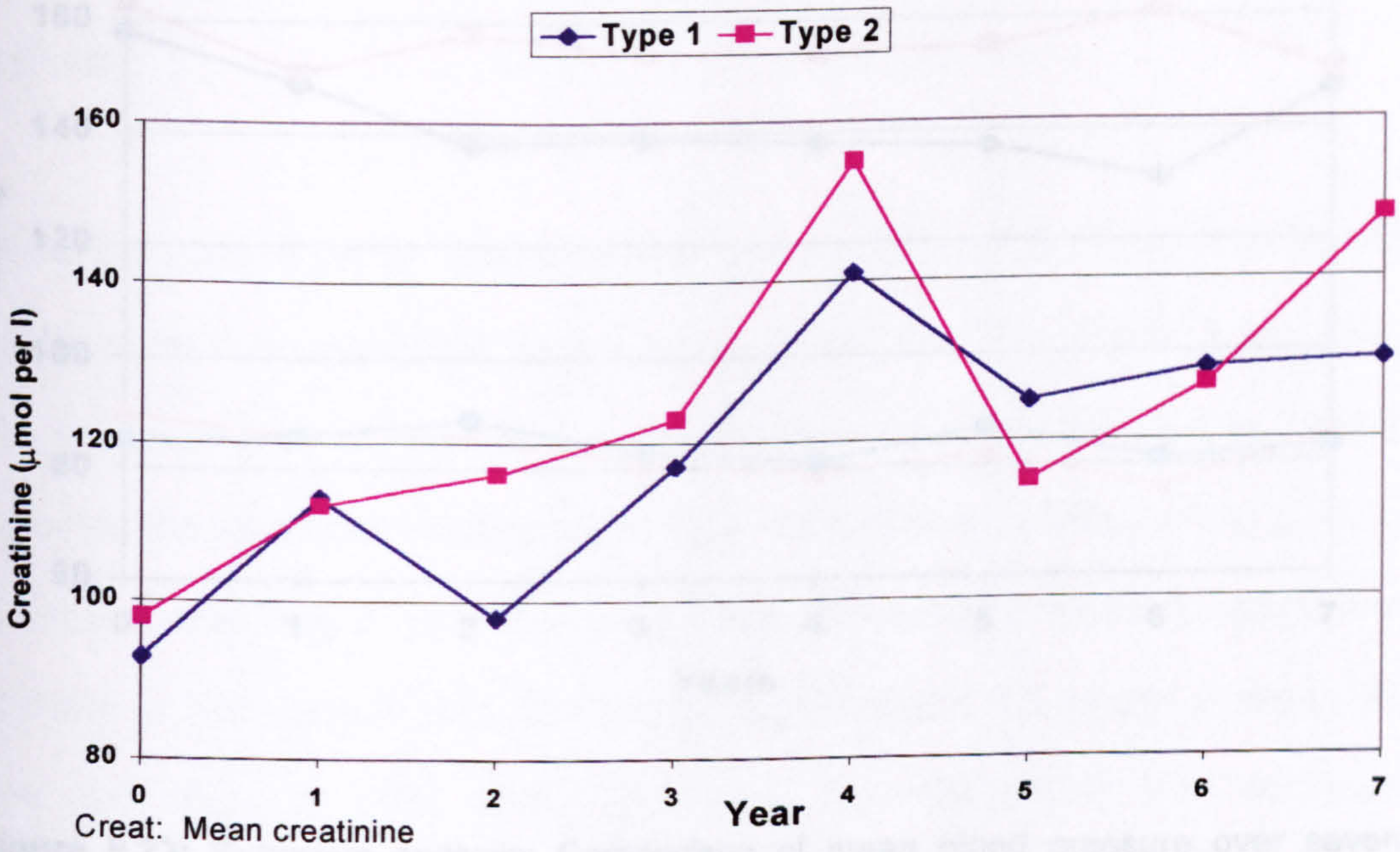

Figure 9.22: Subgroup analysis: Mean serum creatinine concentration over 7 years of follow up

However, when the change between baseline and year 5 was compared, this was not statistically significant. Due to the small number of patients in the Type 2 group from year 5 onwards, further analysis was not performed. The decline in renal function, as assessed by mean inverse creatinine against time, was similar in both groups (*P*=N.S.).

Mean systolic blood pressure at baseline was similar in both groups [Type 1: 158 (SD±28) mm Hg versus Type 2: 163 (SD±30) mm Hg] (*P*=N.S.) (Figure 9. 23).

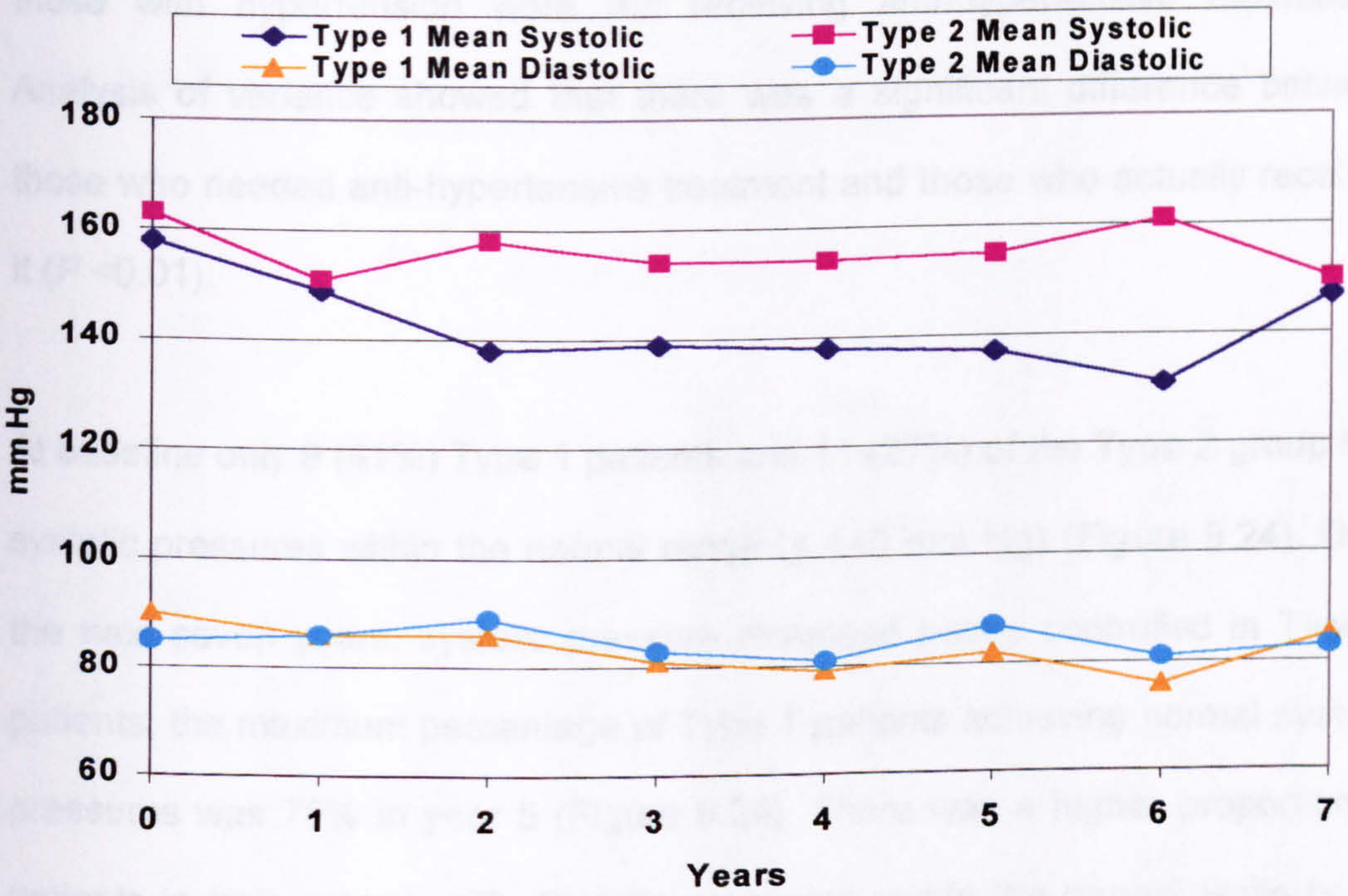

Figure 9.23: Subgroup analysis: Comparison of mean blood pressure over seven years

Analysis of variance showed that there was a statistical difference in systolic pressure between baseline and year 1 in both groups ($P<0.001$). However, there was no difference between baseline up to and including year 5 or between the groups (P=N.S.). There was no statistical difference in diastolic pressure at baseline between the groups (P=N.S.). However, there was a statistically significant difference between diastolic pressure between baseline and year 1 ($P<0.01$) but no difference between baseline up to and including year 5 (P=N.S.). Nineteen (86%) Type 1 and 34 (81%) Type 2 patients were hypertensive at baseline (P=N.S.); 4 (21%) Type 1 and 17 (50%) Type 2 of those with hypertension were not receiving anti-hypertensive medication. Analysis of variance showed that there was a significant difference between those who needed anti-hypertensive treatment and those who actually received it ($P<0.01$).

At baseline only 9 (41%) Type 1 patients and 11 (27%) of the Type 2 group had systolic pressures within the normal range (≤ 140 mm Hg) (Figure 9.24). Over the next seven years, systolic pressure remained poorly controlled in Type 2 patients; the maximum percentage of Type 1 patients achieving normal systolic pressures was 78% in year 5 (Figure 9.24). There was a higher proportion of patients in both groups with diastolic pressures within the normal limits (≤ 90 mm Hg); Type 1: n=13 (59%) and Type 2: n=26 (63%). The minimum percentage of patients in both groups with diastolic pressures within normal limits rose to 70% or above in subsequent years (Figure 9.25).

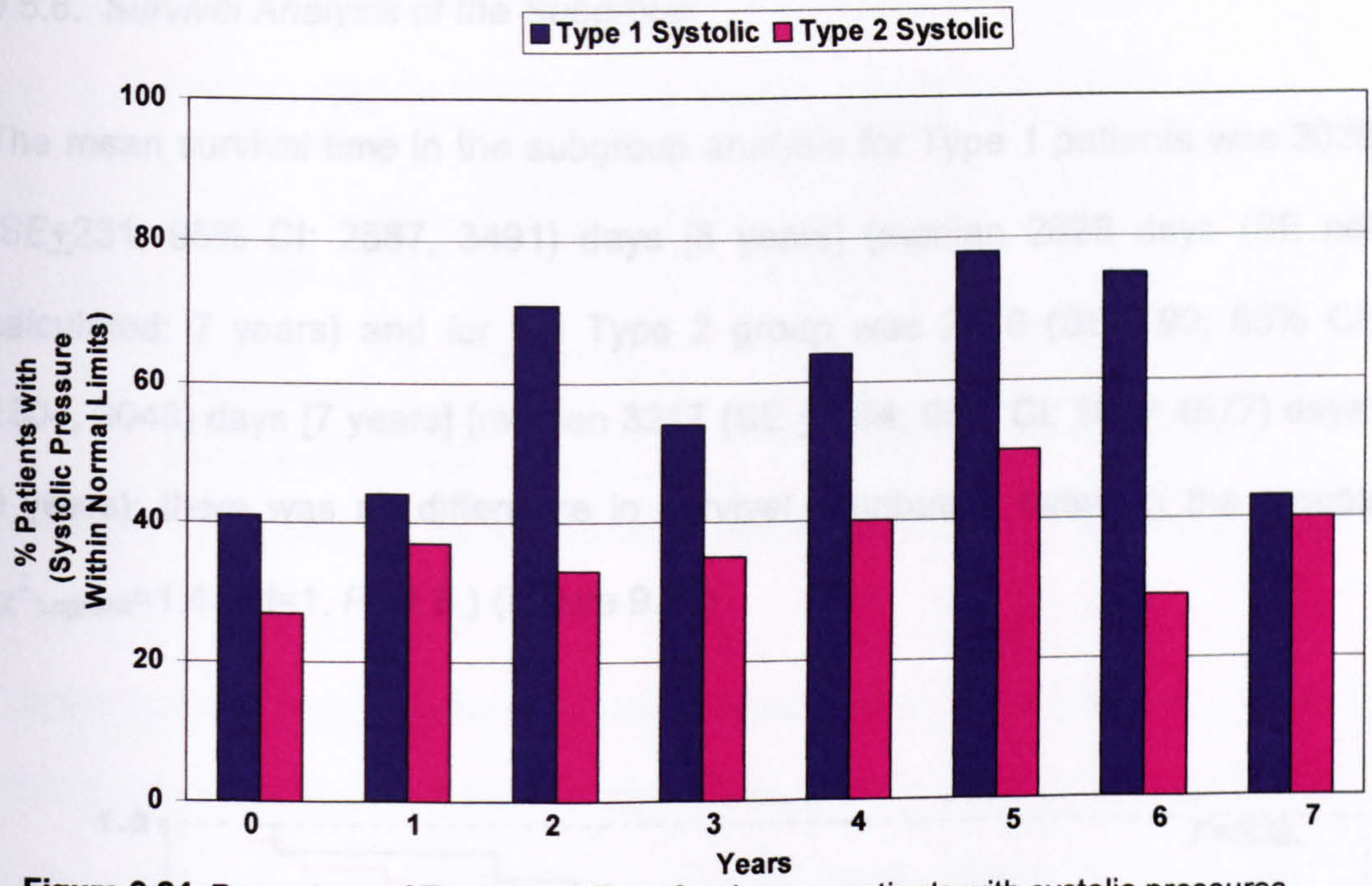

Figure 9.24: Percentage of Type 1 and Type 2 subgroup patients with systolic pressures below the upper limit of normal (<140 mm Hg)

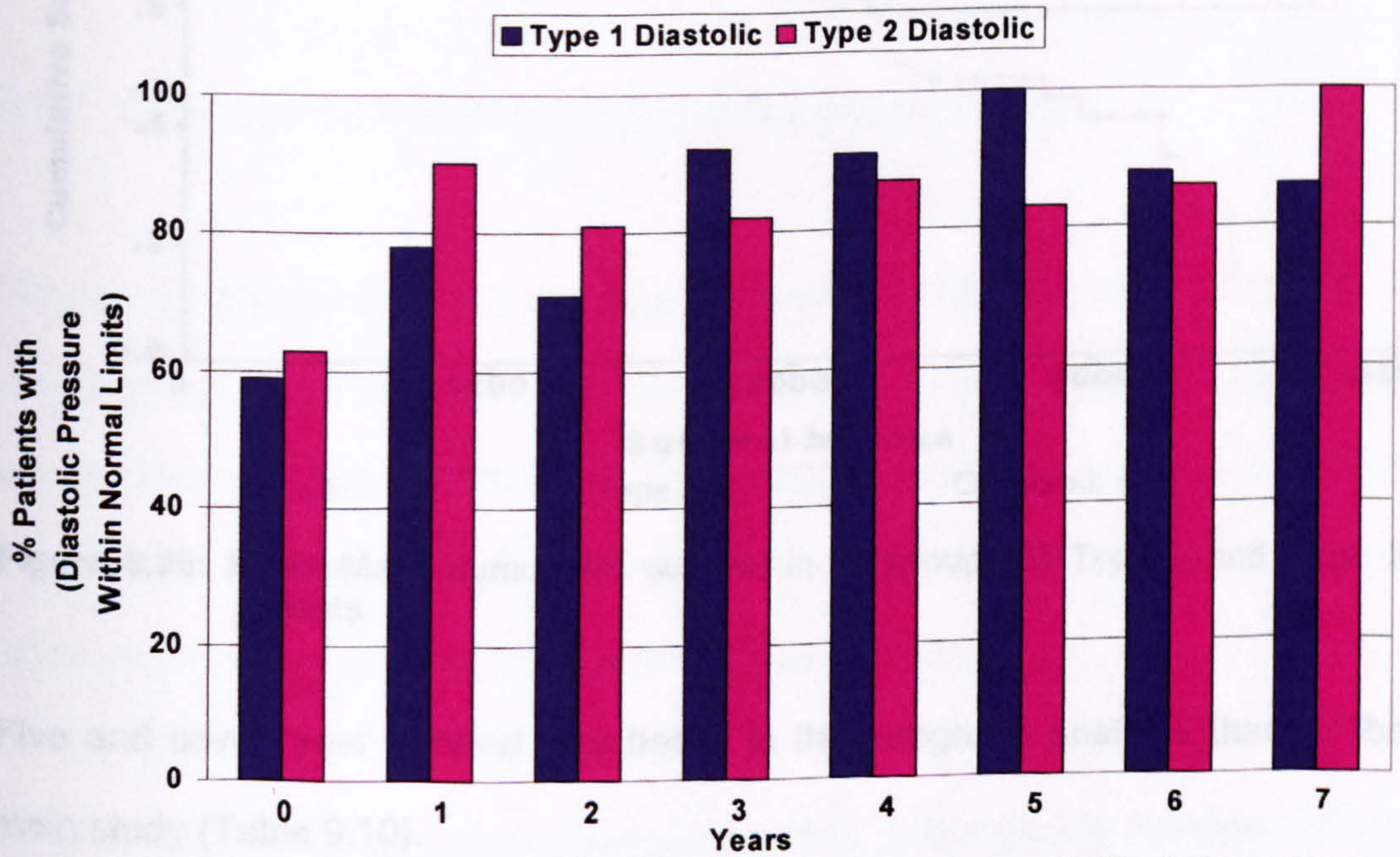

Figure 9.25: Percentage of Type 1 and Type 2 subgroup patients with diastolic pressure below the upper limit of normal (<90 mm Hg)

9.5.8. *Survival Analysis of the Subgroup*

The mean survival time in the subgroup analysis for Type 1 patients was 3039 (SE±231; 95% CI: 2587, 3491) days [8 years] (median 2626 days (SE not calculated: 7 years) and for the Type 2 group was 2676 (SE±190; 95% CI: 2304, 3048) days [7 years] (median 3217 (SE ± 684; 95% CI: 1877,4577) days: 9 years); there was no difference in survival distribution between the groups ($\chi^2_{Logrank}$=1.47,df=1, *P*=N.S.) (Figure 9.26)

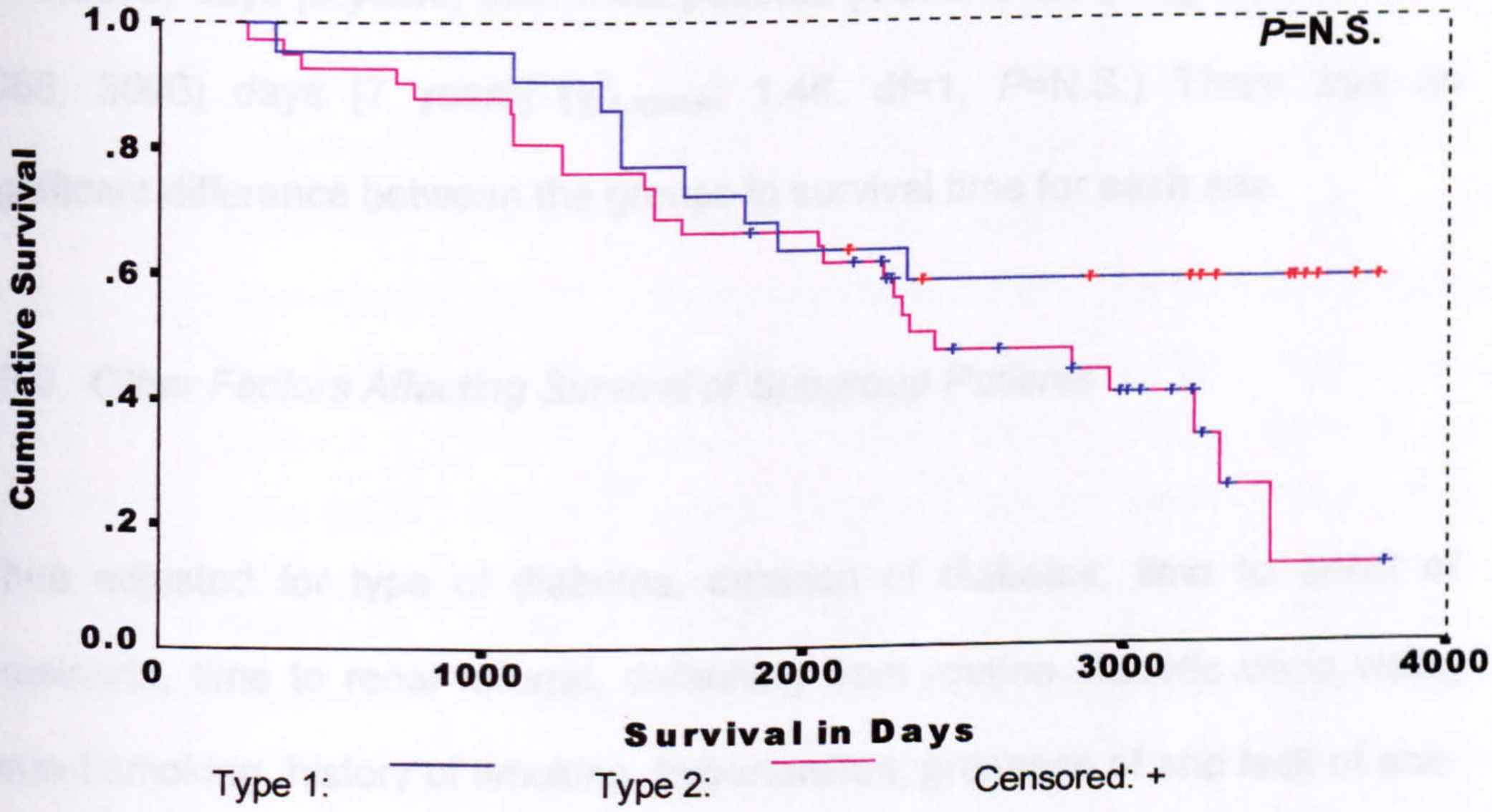

Figure 9.26: Kaplin-Meier cumulative survival in subgroups of Type 1 and Type 2 patients

Five and seven year survival was better in the subgroup analysis than in the main study (Table 9.10).

Type of DM (Group)	Patient Survival		
	5 Year n (%)	7 Year n (%)	10 Year n (%)
1 (Main)	30 (68)	19 (43)	6 (14)
1 (Subgroup)	16 (73)	11 (50)	3 (14)
2 (Main)	43 (44)	28 (29)	3 (3)
2 (Subgroup)	26 (62)	15 (38)	1 (2)

DM: Diabetes mellitus

Table 9.10: Comparison of survival in main and subgroup over 5, 7 and 10 years

Female patients survived for one year longer (mean: 3103 (SE±241; 95% CI: 2630,3575) days [8 years] than male patients (mean: 2727 (SE± 188; 95% CI: 2358, 3096) days [7 years] ($\chi^2_{Logrank}$: 1.46, df=1, *P*=N.S.) There was no significant difference between the groups in survival time for each sex.

9.5.9 *Other Factors Affecting Survival of Subgroup Patients*

When adjusted for type of diabetes, duration of diabetes, time to onset of proteinuria, time to renal referral, defaulting from routine diabetic clinic visits, current smoking, history of smoking, hypertension, presence of and lack of anti-hypertensive therapy in hypertensive patients at baseline and points for blood pressure treatment were not significant factors on survival.

A number of variables were assessed for their influence on survival. Those which were statistically significant or increased the hazard ratio are listed in Table 9.12. Age at referral was a significant factor for survival when adjusted for

type of diabetes (HR=1.1, *P*<0.001) and separately for each group (Type 1: HR=1.1, *P*<0.05; Type 2: HR=1.1, *P*<0.005) (Table 9.11). When adjusted for type of diabetes, serum creatinine at baseline was also a significant factor in cumulative survival (HR=1.1, *P* <0.05), despite all values being within the normal limits (Table 9.11). On individual group analysis, creatinine levels at baseline were significant for survival in Type 1 patients (HR=1.1, *P*<0.01) only.

Type DM	Variable	β	SE	Wald (χ^2)	*P*	Exp β (HR)	95% CI
Adjust TDM	Age	0.1014	0.0262	15.006	0.0001	1.1067	1.0514, 1.1650
Type 1	Age	0.0906	0.0374	5.8577	0.0155	1.0949	1.0174, 1,1783
Type 2	Age	0.1102	0.0351	9.8341	0.0017	1.1165	1.0422, 1.1961
Adjust TDM	Systolic Yr 1	0.0347	0.0123	8.0117	0.0046	1.0353	1.0107, 1.0605
Type 1	Systolic Yr 1	0.0644	0.0230	7.8166	0.0052	1.0665	1.0194, 1.1158
Type 2	Systolic Yr 2	-0.0340	0.0152	5.0233	0.0250	0.9666	0.9383, 0.9957
Type 1	Diastolic Yr 1	0.0679	0.0301	5.0913	0.0240	1.0702	1.0090, 1.1352
Type 2	Diastolic Yr 2	-0.912	0.0344	7.0284	0.0080	0.9128	0.8533, 0.9765
Type 1	Creat. Base	0.0731	0.0280	6.8163	0.0090	1.0759	1.0184, 1.1366
Type 1	Creat. Base +	0.0947	0.0347	7.4432	0.0064	1.0993	1.0270, 1.1767
	Systolic Base	0.0248	0.0156	2.5278	0.1119	1.0251	0.9942,1.0561
Type 1	Hypertension	0.2055	0.5417	0.1439	0.7044	1.2281	0.4248, 3.5510
Adjust TDM	Untreated HT.	0.2354	0.4285	0.3019	0.5827	1.2655	0.5464, 2.9310
Type 1	Untreated HT.	0.2033	0.5408	0.1414	0.7069	1.2255	0.4246, 3.5373
Type 2	Time to Refer.	0.3692	0.1536	5.7537	0.0165	1.4465	1.0699, 1.9558

HR: Hazard ratio TDM: Type of diabetes Yr: Year Creat.: Creatinine concentration
HT: Hypertension Refer: Referral CI: Confidence intervals

Table 9.11: Cox proportional hazards model assessing influence of variables on survival in subgroup analysis.

When creatinine at baseline was added as a covariate to age at referral, neither factor was significant in Type 1 patients although age was still statistically significant in Type 2 patients (HR=1.1, $P<0.005$). Creatinine levels in year 1 were also statistically significant for survival in Type 1 patients (HR=1.05, $P<0.05$) but not in the Type 2 group.

Systolic pressure over six years was assessed on an annual basis for its effect on survival in each of the groups. Systolic pressure at baseline was not a significant factor affecting survival in either group, however, in year 1 it was a significant factor in Type 1 patients (HR=1.1, $P<0.01$) [mean systolic pressure year 1: 149(SD$\pm$25) mm Hg; 5% decrease from baseline mean (four patients had either died or were lost to follow up)] but not in the Type 2 group. In year 2, systolic pressure was significant in Type 2 patients (HR=1.00, $P<0.05$) [mean systolic pressure: 158 (SD$\pm$27) mm Hg; 5% increase from mean in year 1 (nine patients had either died or were lost to follow up)], however, patients with systolic pressures below the mean had a slightly decreased HR (0.97). When systolic pressures from baseline to year 3 were added as covariates, none of these were significant factors on survival. The mean systolic pressure in Type 1 patients decreased to below 140 mm Hg by year 2 and remained below this level until year 7. In contrast, in Type 2 patients the mean systolic pressure remained above 150 mm Hg from year 2 onwards (Figure 9.23). The addition of systolic pressure in year 1 to creatinine concentrations in year 1 as a covariate showed that in Type 2 patients systolic pressure was a significant factor

affecting survival (HR=1.1, $P<0.05$) and that creatinine was not but in the Type 1 group only creatinine was statistically significant (HR=1.1, $P<0.01$) (Table 9.11).

Diastolic pressure over six years was also assessed on an annual basis for effect on survival in each of the groups. Diastolic pressure at baseline, in both groups, was not significant in terms of survival. In year 1, diastolic pressure in Type 1 patients was significant (HR=1.1, $P<0.05$) [mean diastolic pressure: 86 (SD±12) mm Hg; 4% decrease from baseline mean (four patients had either died or were lost to follow up)] but in Type 2 patients it was not. In year 2, diastolic pressure was a significant factor in survival in Type 2 patients ($P<0.01$) [mean diastolic year 2: 81 (SD±13) mm Hg; 2% decrease on mean in year 1 (nine patients had either died or were lost to follow up)], patients with a diastolic below the mean had slightly decreased HR (0.91) (Table 9.11). When diastolic pressure at baseline and in years 1 and 2 were added as covariates, none of these were significant factors in survival in Type 1 patients. However, in the Type 2 group the significance of diastolic pressure in year 3 increased ($P=0.005$) while diastolic pressures in the other two years were not significant. In Type 1 patients the mean diastolic pressure dropped from 90 mm Hg at baseline to 86 mm Hg by year 1 and remained at or beneath this level until year 7. Mean diastolic pressure in Type 2 patients was lower at baseline than in Type 1 patients (85 mm Hg) and by year 2 had gone up to 89 mm Hg and decreased until year 5 (Figure 9.25).

Assessment of systolic and diastolic pressures, as covariates in year 2 showed that in Type 1 patients systolic pressure was a significant factor on survival ($P<0.05$) but diastolic pressure was not. Neither systolic nor diastolic pressures were statistically significant to survival in Type 2 patients. A similar analysis was performed for systolic and diastolic pressures in year 3 which showed that neither parameter was statistically significant for survival in either Type 1 or Type 2 patients.

Glycaemic control (HbA1) was assessed as a factor in survival using data from baseline, years 1-4 pre-baseline and years 1-4 post baseline. When adjusted for type of diabetes, no individual year was a significant factor. However, when years 1-4 pre-baseline were analysed together as covariates, HbA1 in year 3 before referral was a significant factor ($P=0.05$) as was HbA1 in year 4 before referral ($P=0.01$). Mean HbA1 for years 1 to 4 pre-baseline were 12% for each year. Neither the baseline nor post baseline HbA1 readings were significant factors in survival.

9.6 Discussion

9.6.1 *Differences Between Type 1 and Type 2 Patients in Terms of Survival*

In the main analysis, 32% of Type 1 and 56% of Type 2 patients had died by the end of year five of follow up and 86% Type 1 and 97% of Type 2 by the end of year 10. In contrast, the five year survival in the subgroup analysis (patients with baseline creatinine levels within the normal range) was higher with only 27% of Type 1 and 38% of Type 2 patients having died, in Type 1 patients, 10 year survival was the same as the main analysis (86%) and in Type 2 patients 98% had died. Although seven year survival was better in the subgroup analysis (50% of Type 1 versus and 43% in main group and 38% of Type 2 subgroup versus 29% in main group). These results can be compared to the Danish studies described by Parving (1999) where 35% of Type 2 patients with macroalbuminuria had died within 5 years and 44% of Type 1 patients with overt nephropathy had died within 10 years (Parving,1999). In the subgroup analysis, Type 2 survival was similar over five years but in both the subgroup and main studies Type 1 survival was worse over ten years than the Danish studies. However, it is worth noting that numbers of patients in this study were considerably smaller than either of the Danish studies. These results do highlight two important points, firstly the main analysis included patients with advanced renal disease many of whom required RRT or died from ESRF within the first few years of follow up and secondly, that patients with proteinuria and

normal creatinine levels appear to have an increased chance of survival for up to seven years following renal referral but from then onwards there was no difference in survival between the groups. In those patients who received RRT, five year survival was 80% in Type 1 and 50% in Type 2 patients, results which appear to be favourable in comparison to a study in Germany where 40% of Type 1 and 10% of Type 2 patients had survived on RRT for five years and a study in Italy where 5 year survival for all diabetic patients on dialysis was 34% again, the small numbers in the present study may make comparisons unrealistic (Marcelli *et al*, 1996; Ritz *et al*, 1999).

The main analysis has shown that Type 1 patients have a longer mean survival time than the Type 2 group. However, the subgroup analysis has shown that there was no difference in survival time between Type 1 and Type 2 patients. This implies that nephropathy patients with serum creatinine levels above the normal limits have a reduced survival rate in comparison to those with normal creatinine levels. It is also worth noting that a higher proportion of Type 1 patients received RRT and thus survival time may have been extended. Type 1 patients receiving RRT also had a longer survival time than Type 2 patients on RRT, possibly due to more Type 2 patients dying from renal failure, despite receiving dialysis. Female Type 2 patients survived for longer in the main analysis but there was no difference in the survival times between the sexes in either group in the subgroup analysis. This may have been secondary to there

being similar proportions of female patients in each group in the subgroup analysis, unlike the main analysis where there was a preponderance of men.

Type 2 patients in the main analysis were referred more quickly for nephrological assessment than Type 1 patients, but this was not true in the subgroup analysis. There were two explanations for this a) some Type 1 patients had developed proteinuria long before the referral process started plus treatment with ACEI for nephropathy patients started in the diabetic clinic in 1984 therefore Type 1 patients would already have been receiving appropriate treatment and b) that once the referral process was in place, Type 2 patients were referred as soon as persistent proteinuria was diagnosed rather than waiting until the patients' renal function had significantly deteriorated.

The Type 1 group were younger than Type 2 patients in both the main and subgroup analysis. Age at referral was a significant factor on survival in both groups and in both analyses despite the almost twenty year age difference between the groups. Duration of diabetes was longer in the Type 1 group but was not a significant factor on survival in either analysis, unlike the Type 2 group where duration was shorter and was a significant factor on survival in the main analysis only. However, when age was added to duration, duration was no longer a factor in survival but age was. A Japanese study of early-onset Type 2 patients, where the duration of diabetes was longer (mean: 23 years) than patients in this study (mean: 13 years), has shown that duration was a

significant independent factor in survival but this may be secondary to the fact that Type 2 diabetes is common in young Japanese people whose extended duration of diabetes is similar to that of Type 1 patients at the onset of proteinuria (Yokoyama *et al*, 1998). The results of this study also contrast with an age stratified study of survival in Pima Indians with Type 2 diabetes on RRT where Nelson and co-workers demonstrated that duration of diabetes was an independent risk factor for survival (Nelson *et al*, 1996). In the shorter duration Type 2 patients in the present study, there may have been other pre-existing risks i.e.: risk factors for cardiovascular disease which were present even before diagnosis of diabetes.

In the main analysis, creatinine concentrations at baseline were significant in the Type 2 group only, possibly due to the wider range of values than in Type 1 patients, but in the subgroup analysis baseline creatinine levels (within normal limits) were significant only in Type 1 patients despite the close matching of this parameter in both groups. It could be postulated that the younger age of these patients may have been relevant to the significance of serum creatinine levels i.e.: the levels may have been higher than the norm for younger people, however, there was no confirmation of this using the Cox proportional hazard model. In a Chinese study of Type 2 patients, plasma creatinine was found to be a predictive factor for mortality despite the mean value at the start of the study being within normal limits, although the range was wide, however, in those patients who died mean plasma creatinine was raised (Chan et al, 1995).

The comparison of these two studies again highlights the difference between the main and subgroup analysis in this study, reinforcing the advantage for survival in Type 2 patients with normal creatinine levels at baseline.

At referral, the majority of patients (86%) in both groups were hypertensive with one third of Type 1 patients and 55% of Type 2 who needed anti-hypertensive treatment not receiving treatment. Hypertension both contributes to and is caused by renal disease, therefore the need to control blood pressure is all important in delaying the progression of nephropathy and improving survival; the efficacy of various anti-hypertensive drugs in nephropathy patients has been extensively researched (Sawicki *et al,* 1995; Leese and Vora, 1996; Parving, 1999). It would appear despite this knowledge that our cohort of patients were not being treated appropriately. However, the EURODIAB IDDM Complications study has shown that in a large European cohort of Type 1 patients, of those with hypertension only 42% were on anti-hypertensive treatment and that only 11% of those with hypertension had blood pressures which were treated and controlled (Collada-Mesa *et al*, 1999). The authors have suggested that this is due to the lack of understanding that physicians have of the vital importance of controlling blood pressure in patients with diabetes, despite the evidence from research and the clear guidelines on treatment of hypertension in diabetes which exist in many countries.

In this study both groups had systolic hypertension at baseline, although this was not a significant factor in survival. Between baseline and the first year, anti-hypertensive treatment increased which produced a decrease in both systolic and diastolic pressures. However, in the subgroup analysis only, the systolic pressures in year 1 became a significant factor for survival in Type 1 patients. This poses an interesting question because systolic pressure in the Type 1 subgroup remained below the upper limit of normal (140 mm Hg) from year 1 onwards. Possibly, deaths and patients lost to follow up during this first year, may have influenced the significance of systolic pressure by the end of the first year in the Type 1 group. In year 2 having a systolic pressure below the mean value conferred a slightly reduced hazard for survival in Type 2 patients.

In the Type 2 group the mean systolic pressure was above the upper limit of normal throughout the whole study. There was also an increase in both mean systolic and diastolic pressures in the Type 2 subgroup from the initial decrease from baseline to year 1, from year 2 onwards both remained virtually unchanged probably due to some patients who were hypertensive not receiving treatment. However, it could also be that a) anti-hypertensive treatment was not effective, b) dose levels were too low, c) the medical staff were aiming to treat diastolic hypertension without considering systolic hypertension, d) blood pressure was resistant to treatment, or e) that because these were older Type 2 patients the need to aggressively reduce blood pressure was not thought to be as important as in younger Type 1 patients or f) a combination of these factors.

In the subgroup analysis, diastolic pressures were better controlled than in the main analysis and the levels in each group reflected changes in the systolic pressures previously discussed. A number of studies have shown associated systolic hypertension with nephropathy while Vestbo and colleagues have shown that systolic blood pressure was related to urinary albumin excretion in diabetic and non-diabetic subjects (Cooper *et al*, 1987; Klein *et al,* 1988; Nielsen *et al,* 1993; Vestbo *et al*, 1995). These previous studies indicate that Type 2 patients in Wolverhampton are not unique in having raised systolic pressure in connection with nephropathy. The lack of anti-hypertensive therapy at baseline was a significant factor for survival in Type 2 subgroup patients increasing the hazard ratio to 1.5. However, in the Type 1 group, although lack of anti-hypertensive treatment increased the hazard ratio to 1.2, this was not significant; these results also underline the need for aggressive treatment of hypertension in nephropathy but more especially in Type 2 patients.

In the main analysis, other parameters were not significant as independent risk factors for survival i.e.: a history of smoking, defaulting from routine diabetes clinic follow up and HbA1 levels over five years pre- and post referral. Current smoking although it was not a significant factor in survival was a hazard in Type 2 patients.

However, in the subgroup analysis, HbA1 levels at years 3 and 4 pre-referral were significant when adjusted for type of diabetes and in combination with

other pre-referral HbA1 values. It may be possible that the turning point in terms of changes in renal function occurred around three to four years before renal referral especially as the median time between onset of proteinuria and referral was one year in both groups. Patients may have developed microalbuminuria up to three years before developing overt proteinuria. Cooper and co-workers found that in both Type 1 and Type 2 patients the mean duration of the microalbuminuric phase of nephropathy was 3 to 4 years which correlates with the significance of HbA1 in years 3 and 4 pre-referral as a factor in survival in this study (Cooper *et al*, 1987).

In those patients where cause of death was known, a higher proportion of Type 2 patients died from ESRF (24%) than Type 1 (17%). However, 22% of Type 1 patients died from cardiac failure and it would have been useful to know how many of these patients were in renal failure. ESRF results in congestive cardiac failure and it has been reported that many patients in renal failure have cardiac failure recorded in Part 1 of the death certificates (Joint Working Party of Renal Association *et al*, 1989). The large percentage of deaths in each of the groups where the cause was not known highlights the problem of tracking patients' hospital notes following death.

9.6.2 Differences Between Ethnic Groups in Terms of Survival

Type 2 diabetes is more common in Indo-Asian and Black patients than Type 1 and in this study the numbers of Type 1 patients in these two ethnic groups was low (Mather and Keen, 1985; Simmons *et al,* 1989; Cowie *et al*, 1989; Simmons and Powell, 1993; Perneger *et al,* 1994). Several studies have shown that both Black and Indo-Asian patients have an increased risk of developing ESRF. It could be assumed that this is secondary to the higher incidence of Type 2 diabetes in both groups; however, Cowie and co-workers have found a higher incidence of Black patients reaching ESRF even after adjusting for the high incidence of Type 2 diabetes in Black people (Cowie *et al*, 1989; Burden *et al*, 1992; Roderick *et al*, 1994).

In this study, the three ethnic groups were well matched in terms of age, sex, systolic and diastolic pressures and duration of diabetes in Type 2 patients. However, Type 1 White patients had a longer duration of diabetes than either Type 1 Indo-Asian or black patients, which may be secondary to the small numbers of Type 1 patients in the other two ethnic groups rather than to a real difference. Previous studies in Leicester have shown an increased incidence of nephropathy in Indo-Asian men in comparison to Indo-Asian women and White patients which was not confirmed in this study where there was a higher ratio of men: women (2.9) in the White group while the ratio in both Black and Indo-

Asian groups was lower and similar (1.6 and 1.5 respectively) (Burden *et al*, 1992; Koppiker *et al*, 1998).

White patients with Type 1 diabetes survived for longer after renal referral than Type 2 patients but there was no difference in survival in either Indo-Asian or Black patients when type of diabetes was compared. However, there was a significant difference in survival, when adjusted for ethnic group, possibly due to the longer survival time in Type 1 White patients. This longer survival time in Type 1 White patients cannot be explained by some of those patients having received RRT because there was no difference between the groups in mean survival of patients on RRT or between survival in those patients receiving RRT and those not. This increased survival time in White Type 1 patients reflects the poor prognosis for Type 2 patients with nephropathy, as discussed previously. It is worth noting that Cowie and co-workers (1994) have shown in the USA that survival of Black patients on dialysis is longer than that of White patients unlike the present study.

The dramatic difference in survival in Black patients who were current smokers (mean 2 years) in comparison to those who were not (mean 7 years) and the substantially increased hazard ratio (4.6) probably reflects the increased risk of death from cardiovascular disease and in particular from hypertensive disease in Black people, in conjunction with increased mortality risk in these diseases associated with smoking; in the present study 73% of known causes of death in

Black patients were due to cardiovascular disease (Raleigh *et al*, 1996). This view is reinforced by the observations that 100% of Black patients in the present study were hypertensive at baseline, that 56% of those were not receiving anti-hypertensive therapy and that for the first few years of the study Black patients had persistent systolic hypertension (less than 30% had controlled systolic pressures) while 30% also had uncontrolled diastolic pressures.

The significance of serum creatinine concentration at baseline in all three groups, but with hazard ratios close to 1.0, suggests that although serum creatinine was a factor in survival, as expected because these patients were known to have renal disease, in itself it did not contribute a serious hazard. The addition of age to serum creatinine showed that both were factors in survival in White patients although together neither were in Indo-Asian patients and only creatinine was in Black patients although the mean age for all three groups was similar. Interestingly, Cowie and co-workers found that age in Black patients reaching ESRF was higher than in White patients, a finding that would be difficult to extrapolate to the cohort in the present study based on age at referral (Cowie *et al*, 1989). Serum creatinine was lower in Indo-Asian patients for the first two years which may explain the decrease in significance of this factor when age was added.

Despite the similarities in mean proteinuria at baseline, proteinuria was a more significant factor in survival in Indo-Asian patients than in Black patients, with increased hazard ratios in both, but was not significant in White patients. When this is considered, along with the lower mean serum creatinine levels in Indo-Asian patients at baseline and the significance of time to renal referral after development of overt proteinuria as an independent factor in survival in this group only (despite the mean time to referral being similar in all three groups), these factors suggest that proteinuria is a major risk factor in Indo-Asian patients in comparison to the other two groups. The increased risk posed by proteinuria may be related to the increased prevalence of nephropathy, previously demonstrated in this ethnic group (Gujral *et al*, 1997).

The inability to speak English in Indo-Asian patients, a factor which may considerably disadvantage patients in terms of understanding how to monitor and care for their diabetes, was a significant hazard for survival especially in men. This was not surprising as many Indo-Asian patients in Wolverhampton neither speak nor read English (Wilson *et al*, 1993). It was perhaps not surprising that it was more of a hazard in men than women, given that 83% of male patients in comparison to 60% of females who died did not speak English.

9.6.3 Comments on the Methodology

This study was based entirely on normal clinical practice without any predetermined criteria for commencing anti-hypertensive therapy at specific blood pressure levels. Instead, physicians made decisions based on the patients condition at each clinic visit (as they normally would). This resulted in a useful insight into how blood pressure control is managed in nephropathy patients over a number of years in a clinical setting. However, a protocol detailing examination techniques, biochemical investigations and the levels of diastolic and systolic blood pressure at which treatment should be instigated as well as anti-hypertensive drugs and dosages may have improved blood pressure control, assuming that declining renal function was not the reason that hypertension was not controlled, it is likely that this would also have improved survival as other research has shown (Rossing *et al*, 1996).

The difficulty in locating the medical records of patients who had died resulted in a distinct lack of information on the cause of death in many patients. This could have been avoided if the patients' records had been marked in advance as being part of a research project.

The relatively small number of patients in this study provide information on survival in people with nephropathy in Wolverhampton but made it difficult to compare these results with results from much larger studies elsewhere.

9.7 Conclusions

1 Early referral of patients with proteinuria and serum creatinine concentrations within normal limits appeared to increase seven year survival in both Type 1 and 2 diabetic patients with nephropathy. However, this may reflect the natural progression of nephropathy in that people with serum creatinine levels within the normal limits would take a longer time to reach end stage renal failure than those with creatinine concentrations above the upper limit of normal.

2 Five year survival on RRT was better than in other studies in both types of diabetes.

3 Age at referral in both the younger Type 1 and the older Type 2 patients was a significant factor in survival.

4 Creatinine concentration at referral, even when within normal limits, was a significant factor for survival in Type 1 patients (HR=1.1).

5 Untreated hypertension at renal referral increased the risks for survival in Type 1 patients (HR=1.2).

6 Blood pressure levels over the two years after referral were significant for survival in patients with creatinine concentrations within normal limits at referral.

7 In Type 2 patients systolic hypertension remained uncontrolled throughout the entire follow up period.

8 Type 1 White patients survived for longer than White Type 2 patients.

9 Current smoking was a significant risk to survival in Black patients (HR=4.6)

10 There was no difference in survival time following RRT between the ethnic groups.

11 Black patients had the worst controlled systolic hypertension.

12 Proteinuria at baseline was a hazard for survival in Black patients (HR=1.2).

13 Indo-Asian patients had lower serum creatinine levels for the first two years, had an increased risk to survival from proteinuria levels at baseline (HR=1.3) and from the length of time to renal referral after

developing persistent proteinuria (HR=1.3). These suggest a major difference in nephropathy in Indo-Asian patients which cannot be further explained by this study.

14 The inability to speak English was a significant hazard to survival in Indo -Asian patients (HR=1.7), especially in men (HR=2.6).

9.7.1 Summary of Conclusions

1 Routine follow up of patients in a nephrology clinic appeared to improve survival in Type 1 and 2 nephropathy patients with serum creatinine levels within the upper limit of normal, however, without performing a controlled trial to assess survival in patients with the same creatinine concentrations either attending a nephrology clinic or not, it would be inappropriate to either accept or reject $H_0(1)$.

2 Survival time was not the same in patients from different ethnic groups, therefore, accept $H_1(2)$.

3 There was a difference in factors which affect survival in Type 1 and 2 nephropathy patients as well as similarities, therefore, accept H_1 (3).

4 There was a difference in factors which affect survival in patients from different ethnic groups, therefore, accept H_1 (4).

Chapter 10

Review of the Thesis

10.1 Novel Aspects of the Research

This project specifically studied nephropathy in the diabetic population of Wolverhampton, a subject which has not been researched previously in this town. Wolverhampton has a mixed ethnic population, with a higher percentage of Indo-Asian (12%, 1995) than Black people (5%, 1995), high levels of unemployment and social deprivation [using the Townsend score of material deprivation: 35% of the wards in Wolverhampton had the highest Townsend score i.e.: severe deprivation, with only 10% having a score in minus figure i.e.: an indication of affluence (Hutchby *et al*, 1996)].

All of these factors contribute to poor health and an increased burden on health care services in that Indo-Asian people have a higher incidence of coronary artery disease, diabetes and nephropathy than the indigenous population, Black people have a high incidence of diabetes, nephropathy and a higher death rate from hypertension related causes, while low income and social deprivation can lead to poor nutrition, amongst many other things, which in turn may exacerbate the difficulties in controlling diet in people with diabetes (Allawi *et al*, 1988; Odugbesan *et al*, 1989; Cowie *et al*, 1989; Burden *et al*, 1992; Perneger *et al*, 1994; Raleigh *et al*, 1996; Shaukat, 1995; Gujral *et al*, 1997). Thus in Wolverhampton, the population is potentially more at risk of developing both diabetes and nephropathy than in an affluent town with a predominantly White employed population. Added to this are the difficulties in communicating with

Indo-Asian patients who neither speak nor read English and the cultural differences between White and Indo-Asian and possibly Black patients which influence the approach and perceptions of each of these ethnic groups to diabetes (Wilson *et al*, 1993; Greenlaugh, 1997). Other places with populations containing substantially sized ethnic groups have been well researched in terms of diabetes (Coventry and Southall) and nephropathy (Leicester) but this has not been done previously in Wolverhampton (Mather and Keen, 1985; Simmons *et al*, 1989; Gujral *et al*, 1997).

The data collected for this project reflected normal clinical practice rather than a formal research protocol. A research project performed within the limitations of a structured protocol detailing methods of examination, upper limits of blood pressure for treating hypertension etc. would have provided results which would certainly have differed from those obtained; there is individual variation in the decision-making processes of clinicians during normal clinical practice in comparison to decisions made when implementing a structured research protocol. The evidence provided by this project on the lack of anti-hypertensive therapy received by nephropathy patients in Wolverhampton has highlighted the difficulties of maintaining blood pressure control within a clinical setting despite the widespread knowledge that effective anti-hypertensive treatment can delay the progression of nephropathy (Mogensen, 1982; Parving *et al*, 1983). It was reassuring that in the EURODIAB study the same lack of effective hypertension management was observed across Europe and that this was not a problem

specific to Wolverhampton (Collada-Mesa *et al*, 1999). However, these results, which cover eight years of treatment, have identified an area of clinical care that needs to be addressed and improved.

The concept of combined diabetes and nephrological clinical management of patients with nephropathy was new in Wolverhampton in 1987 and the medical care provided by this combined approach has been assessed during this project. Combined clinical care has been encouraged by both diabetic physicians and nephrologists with the belief that it would lead to improved prognosis for nephropathy patients. However, in this study, the overall benefits in terms of survival were similar to previous reports, primarily due to the difficulties in controlling hypertension.

The study of nephropathy in different ethnic groups, although only a "snap-shot" of the problem due to the small sample sizes, has identified that difficulty in speaking English in Indo-Asian patients is linked to defaulting from routine diabetic clinic follow up, the presence of retinopathy, raised diastolic pressure and ultimately the risk of death from nephropathy. The results of the present study highlight the need to provide health care which can meet the needs of all people in the population by ensuring (a) adequate numbers of health care professionals who speak the languages of minority groups and (b) that health promotion/education is presented in languages that people understand.

10.2 This Research in the Overall Perspective of Research into Diabetic Nephropathy

The study of mortality in diabetic and non-diabetic people in Wolverhampton aimed to provide an insight into the conditions which were responsible for deaths in both groups of people and to place nephropathy, as a fatal complication of diabetes, into context within the Wolverhampton diabetic population. The increased risk of death from cardiovascular disease in diabetic women in comparison to diabetic men and non-diabetic women found in the present study has been identified in previous research (Kannel and McGee, 1979; Moss *et al*, 1991). An interesting aspect of this part of the study was the consistently high mortality over ten years from cardiovascular disease in non-diabetic people in Wolverhampton, results which reflect the high levels of cardiovascular disease in the general population (Kelleher *et al*, 1994; Hutchby *et al*; 1996). Diabetic nephropathy accounted for only 4% of deaths in diabetic people but it is likely that patients with nephropathy died from cardiovascular disease as has been shown in other research (Wong *et al*, 1991).

One aim of this research project was to identify factors which were associated with diabetic nephropathy in both Type 1 and Type 2 patients from information routinely collected during medical consultations at out-patient clinic visits. An enormous amount of research has been performed on diabetic nephropathy; an initial literature search using Medline identified thousands of references to this

topic. Most of these studies have been in Type 1 patients and many risk factors for nephropathy in this group have been identified; predominantly poor glycaemic control, the presence of microalbuminuria, raised blood pressure, duration of diabetes and a high incidence of microvascular complications (Andersen *et al*, 1983; Parving *et al*, 1983; Parving *et al,* 1988; Vora, *et al*, 1993; Mathiesen *et al*, 1995). The present study was designed to identify any other factors associated with nephropathy in Type 1 patients and specifically to study Type 2 nephropathy patients in depth.

This research has demonstrated that a history of smoking was associated with the presence of nephropathy in both Type 1 and Type 2 patients compared to control subjects, similar to the results of other studies (Microalbuminuria Collaborative Study Group, UK, 1993; Chaturvedi *et al*, 1995; Bruno *et al,* 1996). Defaulting from routine diabetic clinic visits was also a factor associated with nephropathy in both Type 1 and Type 2 patients in comparison to controls and to Type 2 patients with non-diabetic renal disease; confirming the results of previous research in Wolverhampton which showed that defaulting from clinic was associated with all microvascular complications (Hammersley *et al*, 1985). The present study of Type 1 patients compared to control subjects identified the known risk factors: poor glycaemic control, the presence of retinopathy and hypertension but did not identify any unknown factors associated with nephropathy. This was not surprising as nephropathy in Type 1 patients has been studied extensively for over two decades and factors associated with

nephropathy which could be identified using observational data have been documented in previous research (Andersen *et al*, 1983; Parving *et al*, 1983; Parving *et al*, 1988; Mathiesen *et al*, 1995). Ongoing and future research into the molecular and genetic causes of nephropathy in Type 1 diabetes will produce new data that will hopefully lead to ways of preventing or at least effectively treating nephropathy.

Over the last decade interest has grown in nephropathy in Type 2 patients probably due to the increasing numbers of people developing Type 2 diabetes throughout the world and to the higher numbers of Type 2 patients surviving long enough to reach end stage renal failure (Ritz and Stephanski, 1996). When the present research started there was less known about nephropathy in Type 2 diabetes than there is now. Similarities in the factors associated with nephropathy in Type 2 patients from different countries have been demonstrated (Italy, Korea and, apart from the earlier age of onset of Type 2 diabetes, in Japan) (Bruno *et al*, 1996; Park *et al*, 1998; Tanaka *et al*, 1998). However, different ethnic groups with Type 2 diabetes are more susceptible to nephropathy than others (Pima Indians, Indo-Asians and Aborigines) (Ritz and Stephanski, 1996). This suggests that the factors associated with nephropathy are only part of the problem and that unknown genetic factors may be responsible for the development of nephropathy in Type 2 as well as in Type 1 diabetes.

In the present study, Type 2 nephropathy patients had more retinopathy and peripheral neuropathy than control subjects and patients with non-diabetic renal disease, therefore, the presence of microvascular complications was associated with nephropathy in Type 2 patients similar to Type 1 patients (Parving *et al*, 1988). Age and duration of diabetes were both predictive of renal function in Type 2 nephropathy patients. The presence of hypertension was a risk factor in this group and in addition increased body mass index was associated with untreated hypertension.

There were fewer Indo-Asian and Black Type 2 patients with non-diabetic renal disease which may suggest that ethnicity was also a factor associated with nephropathy. However, previous research has shown that both Indo-Asian and Black people have a high incidence of non-diabetic as well as diabetic renal disease (Perneger *et al*, 1996; Gujral *et al*, 1997). The onset of retinopathy and hypertension at an early duration of diabetes in Indo-Asian patients was interesting in that the exact duration of diabetes in Type 2 patients before diagnosis is never known and many Type 2 patients present with established microvascular complications (UK Prospective Diabetes Study Group, 1994). It could be assumed that for some reason, possibly cultural, that Indo-Asian people take longer to have their diabetes diagnosed than White and Black people. However, so far there is no evidence of this and there may be a genuine difference in the progression of microvascular complications in Indo-Asian people which requires further investigation.

Early referral of patients with proteinuria and serum creatinine concentrations within the upper limit of normal appeared to improve seven year survival in this group, although this may have reflected the natural progression of the disease in these patients. In addition five year survival of patients on RRT was better than in other studies (Marcelli *et al,* 1995; Ritz *et al,* 1999).

The positive effects on renal function in nephropathy patients of controlling hypertension are well documented (Mogensen, 1982; Parving *et al*, 1983; Sawicki *et al*, 1995; the EUCLID study group, 1997; Mogensen, 1999). In this study, uncontrolled systolic hypertension was present in both Type 1 and Type 2 patients at renal referral and in many patients during the follow up period; hypertension was also identified as a hazard to survival. Many patients with hypertension were not receiving anti-hypertensive medication at nephrological referral. Initial attempts at controlling hypertension in the first year following referral improved blood pressure levels but this was not sustained in some patients possibly due to declining renal function. Therefore, treatment of hypertension as a preventative strategy prior to and following renal referral was not as effective as it could have been.

Future preventative strategies in Type 1 and Type 2 patients should include aggressive anti-hypertensive treatment of (a) all diabetic patients to prevent the progression of nephropathy and other complications of diabetes and specifically (b) Black and Indo-Asian patients before the onset of proteinuria as proteinuria

was shown to be a hazard for survival in both of these groups. Aggressive anti-hypertensive treatment should occur as soon as any level of proteinuria is detected on "Albustick" testing for both Type 2 and Type 1 patients, especially as creatinine concentration at referral was a hazard even in Type 1 patients with "normal" serum creatinine levels. These are pragmatic suggestions as the ideal would be to routinely test for microalbuminuria and start anti-hypertensive treatment when microalbuminuria is detected. This routine testing is presently not performed in the diabetic clinic and is unlikely to be started due to financial constraints.

Finally, Indo-Asian patients must be regarded as an "at risk" group due to (a) the increased risk of developing nephropathy and (b) to survival posed by not speaking English and the apparent impact this has had on their attendance at the diabetic clinic. Based on the lack of knowledge about the symptoms of diabetes found by Jackson and colleagues (1991), a town wide health education programme, presented in ethnic languages with the aim of informing Indo-Asian people about the symptoms of diabetes, and encouraging them to proactively care for their own health along with readily available language translation facilities in hospital, may reduce the risk of developing nephropathy and other complications that not speaking English confers in this group.

10.3 Proposed Future Research

10.3.1 A Multicentre, Randomised, Controlled Study of the Management of Hypertension in Diabetic Nephropathy

Background: The effective management of hypertension in patients with microalbuminuria can slow down the progression of renal disease (Ravid *et al*, 1996; Mogensen, 1999). Patients with overt proteinuria (but not in end stage renal failure) should also benefit from an aggressive approach to controlling blood pressure as hypertensive damage to the kidneys compounds the disease process already caused by nephropathy. The present study has shown that routine clinical management of hypertension in nephropathy is not as consistent or effective as it could be. The proposed study assesses the efficacy of a structured approach to the management of hypertension in nephropathy patients in comparison to normal clinical practice. A multicentre study is required as the numbers of patients with nephropathy in one diabetic clinic would not be sufficient to provide statistical power for the study.

Aim: To assess the efficacy of a structured interventive method of managing hypertension in diabetic patients with nephropathy by means of a multicentre, randomised, controlled study.

Objectives: To compare a structured interventive approach to the management of hypertension with the normal clinical management of hypertension in patients with diabetic nephropathy over a ten year period.

Patients and Methods:

Definitions:

Proteinuria (>200 $\mu g\ min^{-1}$) as determined by presence of urinary albumin excretion (AER) measured by radioimmunoassay of samples from two overnight urine collections (American Diabetes Association, 1997).

Hypertension: Systolic pressure >130 mm Hg and diastolic >85 mm Hg determined as the mean of two sitting blood pressure measurements using a digital sphygmomanometer (more accurate than the random zero variety) (American Diabetes Association, 1997).

Type 1 diabetes: Diagnosis before 35 years of age requiring insulin treatment.

Type 2 diabetes: Diagnosis after 35 years of age requiring oral or diet therapy for at least one year after.

Normal serum creatinine concentration: 60-120 $\mu mol\ l^{-1}$ as measured by the Jaffe reaction.

Patients:

Inclusion Criteria:

1) All Type 1 and Type 2 diabetic patients with established nephropathy i.e.: proteinuria on measurement of AER, with normal serum creatinine concentrations and blood pressure levels > 130/85 mm Hg.

2) Presence of diabetic retinopathy

3) Confirmation of diagnosis of diabetic nephropathy on renal biopsy in the absence of diabetic retinopathy.

Exclusion Criteria:

1) Serum creatinine concentrations above 120 μmol l^{-1}.
2) History of hypertension prior to onset of microalbuminuria or overt proteinuria.
3) Evidence of raised blood pressure only during clinic visits (as measured be 24 hour blood pressure monitoring).
4) Evidence of renal disease due to causes other than nephropathy.
5) Evidence of hypertension due to other causes.

Methods:

Patients will be screened for proteinuria by measuring AER using two overnight urine saves. Creatinine concentrations, blood pressure measurements (x2 in sitting position and 24 hour blood pressure monitoring x 1; to exclude patients with "white coat syndrome") and assessment of inclusion and exclusion criteria

will be performed at screening. The screening visit will be the baseline measurement for the above parameters.

Patients meeting the inclusion criteria will be entered into the study or control group depending on randomisation. Centralised randomisation will be performed following development of a system for randomisation using random number tables. Each diabetic clinic will be randomised to be either in the study group or the control group in order to reduce any potential alterations to normal clinical management of hypertension which could occur if some patients in the clinic were in the study group and others were in the control group.

There will be two separate methods used in this study. The control group will receive the normal clinical management of hypertension provided in individual diabetic clinics i.e.: at the discretion of the physician, this will include normal clinic follow up times and the usual approach to hypertension management for each clinic. At clinic visit, details of two sitting blood pressure measurements, anti-hypertensive therapy and serum creatinine concentrations will be assessed as well as glycaemic control (HbA1c). Each year AER (assessed from two overnight urine saves) will be determined after the initial baseline assessment.

The study group will receive an interventive approach to hypertension management. As soon as blood pressure levels are detected above 130 mm Hg for systolic or 85 mm Hg for diastolic pressure, anti-hypertensive medication will

commence. In Type 1 patients first line treatment will be an ACEI, with the dosage increased until blood pressure returns to ≤130/85 mm Hg. If the maximum dosage of ACEI does not achieve this target, treatment may be supplemented by a calcium channel antagonists or other anti-hypertensive therapy to achieve the target blood pressure. In Type 2 patients ACEI may be used as previously described in suitable patients but renal function should be assessed very regularly or calcium channel antagonists should be used as first line treatment. Other anti-hypertensive medication may be used instead of or to supplement the first line treatment to reach either a target blood pressure of ≤130/85 mm Hg or if original systolic pressure is ≥180 mm Hg to achieve a reduction in systolic to 160 mm Hg or if systolic pressure is between 160-179 mm Hg to lower this by 20 mm Hg (American Diabetes Association, 1997).

Patients will have regular assessment of blood pressure at nurse led clinics e.g.: every four weeks until blood pressure levels fall to ≤ 130/85 mm Hg. The initial prescription of anti-hypertensive medication will be made by the physician and subsequent increases in dose may be performed by the nurse using a protocol for treatment which has been approved by the Study Steering Committee. If nurse prescribing were approved by the United Kingdom Central Council for nursing, this would facilitate hypertension control in a diabetic clinic setting and in this study. However, nurse prescribing in the UK is still in its infancy with only limited prescribing by a relatively few number of nurses.

The study group will continue to be assessed at visits every eight weeks once blood pressure control has been achieved. Serum creatinine concentrations will be measured every six months. Following the baseline measurement, AER (assessed from two overnight urine saves) will be determined every six months through out the study.

Outcomes:

The following outcomes will be assessed in each group: effective maintenance of blood pressure control, renal function, time to reaching end stage renal failure and survival. The interventive treatment in the study group and normal clinical practice will be compared to determine if the interventive approach is more effective in (a) controlling hypertension, (b) delaying the decline in renal function (assessment of AER) (c) prolonging survival.

Analysis:

Descriptive statistics will be performed. Chi^2 tests will be used to compare categorical data. Comparison of blood pressure measurements, AER, serum creatinine levels and HbA1c at baseline will be made using Student t-test or Mann-Whitney U test (if appropriate). Subsequent comparisons of these parameters (i.e.: over the following years) will be made using analysis of variance. Percentage differences in numbers of patients with controlled hypertension, reaching end stage renal failure and dying in each group will be

assessed using Chi^2. Kaplin Meier survival curves will be used to show differences in survival between the groups.

Based on the results of a study by Sawicki and co-workers (1995) where they achieved 2% reduction in systolic pressure over 5 years of intensified hypertension control in the study group of Type 1 patients (n=34) in comparison to 11% increase in systolic pressure in the control subjects (n=25) ($P<0.01$), in order to detect a reduction of 10% in systolic pressure with a power of 80% and a statistical significance level of $P \leq 0.05$, 199 patients would be needed in each group in this study (i.e.: 2 groups of Type 1 patients [study and control] and 2 groups of Type 2 patients [study and control]).

Study Management:

The study will last for a maximum of ten years to give an accurate indication of the effects of the interventive strategy on survival. Interim analysis of results will be undertaken at years 2, 5, and 7 to determine the effect of the interventive strategy on blood pressure and AER. If results indicate that either of those parameters in patients in the control group are poorly controlled, to the detriment of potential patient survival, and in comparison to the study group, the study may be terminated early to allow control subjects to receive more intense blood pressure control, although follow up of patients will be needed to asses renal and survival outcomes. A Steering Committee of investigators and experts in the field

will be formed to monitor the progress of the study with the power to prematurely terminate the study if this is appropriate.

A co-ordinating centre will be established to manage the study, collect all data from centres and to monitor and validate the data. A study co-ordinator will be appointed to act as primary contact for the study and to liase with the study centres, the Steering Committee and ethical committees.

Ethical Considerations:

The study will be performed in accord with the guidelines of the Declaration of Helsinki (1996 revision). As this is a multicentre study, approval will be sought from a Multicentre Regional Ethics Committee in the first instance and then from the Local Research Ethics Committee for each centre.

Patients will be required to give fully informed written consent prior to the screening visit. Information about the study will be provide orally and in writing and Patient Information leaflets will be produced for this purpose. Confidentiality will be maintained by use of patient identification codes rather than using patients names.

The study will be conducted to the standards of the ICH good clinical research practice guidelines. Monitoring and data validation will be performed by the co-ordinating centre.

10.3.2 Outline of a Study to Determine the Natural History of Diabetic Nephropathy in People from Different Ethnic Groups

Aim: To determine whether there are specific differences in the onset and progression of nephropathy in Indo-Asian, Black and White Type 2 diabetic patients.

Objectives:

(1) To identify all new cases of Type 2 diabetes in several centres with large populations of Indo-Asian and Black patients over a period of one year.

(2) To follow the progress of patients in each ethnic group over a minimum period of fifteen years.

(3) To determine the onset of microalbuminuria, retinopathy and hypertension in patients in each ethnic group.

(4) To monitor the progression of nephropathy in patients in each ethnic group.

(5) To determine the quality of life of patients with newly diagnosed diabetes and in subsequent years.

Study Design:

A multi-centre, cohort study of Type 2 diabetic patients followed-up for a minimum of fifteen years into which all newly diagnosed Type 2 patients would be entered unless they had evidence of microvascular complications at diagnosis

of diabetes (including microalbuminuria). The power of the study would of necessity be large enough to identify small differences between the groups.

Methods:

Patients would attend for study visits once/year in addition to normal diabetic clinic visits. Details of ethnicity group, place of birth, gender, age, dietary type and whether English was spoken would be recorded at the start of the study.

At each visit the following parameters would be assessed:

Sitting blood pressure x 2

Microabluminuria / Proteinuria status

Albumin excretion rate (timed overnight urine saves)

Serum creatinine concentration

HbA1c

Diabetic, anti-hypertensive, lipid lowering and other treatments would be recorded (including dosages, changes to dose and treatment and if possible compliance).

Assessment of diabetic complications

Quality of life assessment (once/year).

Determination of language/s spoken, regular attendance at clinic visits, smoking habits, cultural and social differences i.e.: religion, country of birth, employment status and dietary habits.

Statistical Analysis:

Incidence will be determined. Analysis of variance will be used to assess between and within group differences. Chi^2 tests will be used to assess differences between categorical variables. Correlation and linear regression will be used to determine relationships between different variables. Multiple and logistic regression will be used to assess the effects of multiple variables on specific parameters. Relative risk of developing nephropathy and other end points will be determined. Survival analysis will be performed if appropriate.

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Appendix 1

Point System Determined for Anti-hypertensive Therapy

A1 Introduction

A small pilot study was performed to determine changes in anti-hypertensive drugs and dosages defined as a point scale, the aim of which was to produce a quantitative method for assessing levels of treatment based on type of medication and dosage.

A2 Methods

Five consultant physicians were asked retrospectively to complete a questionnaire (A2.1) on preferred drugs and dosages for the treatment of hypertension in Type 1 and Type 2 patients with nephropathy. The consultants were asked to assess a range of different anti-hypertensive drugs and allocate points on a scale from 1-10 (10 being the most preferred treatment) dependent on whether they were treating Type 1 or Type 2 patients. Points allocated by each consultant for type of drug were added together, the total was then averaged by dividing by the number of consultants. The drugs were subsequently graded according to treatment preference i.e: grade 1 was the most preferred. Finally, points were allocated with the highest points equalling the highest grade e.g.: Grade 1=22 points, Grade 22=1 point (Tables A3.1-A3.3).

The consultants were also asked to allocate points for dosages of these drugs again on a scale from 1-10 (10 being the most preferred dosage for maximum

effect) depending on type of diabetes. However, as only two consultants completed this section of the questionnaire, an average was determined for those drugs chosen as preferred drugs by both consultants. As one consultant had allocated points, as requested, to each dose of each drug, this information was used to determine points for the remaining drugs and dosages for each patient from baseline (at renal referral) until the end of the follow up period.

A2.1 Questionnaire

Instructions

I am trying to develop and validate a point system for determining the effectiveness of anti-hypertensive medication in patients with diabetic nephropathy. I have listed the different drugs and dosages which patients have been treated with. I would like you to:

A Award each dose of each drug a point between 1-10 depending on your own clinical judgement of the effectiveness of the dosage.

B Comment in the preference section which dose you would be likely to prescribe initially in patients with:

a) Systolic =140-160 mm Hg + Diastolic = 90-110 mm Hg

b) Systolic>160<200 mm Hg + Diastolic = 90-110 mm Hg

c) Systolic > 160<200 mm Hg + Diastolic >120 mm Hg

d) Systolic > 200 mm Hg + Diastolic =120 mm Hg

Please mark comments with a), b), c) and d).

C Comment on which type of drugs are your preferential and second choice of treatment *in* Type 1 patients with nephropathy and Type 2 *patients with nephropathy.*

Scoring System for Hypertension Treatments			
Drug	**Total Daily Dose**	**Point**	**Preference in Diabetic Nephropathy**
Atenolol (mg)	25 50 75 100		
Bisoprolol (mg)	5 10		
Celiprolol (µg)	200 300 400		
Frusemide (mg)	20 40 80 120 160		
Bumetamide (mg)	1 2 3 4 5 6		
Indapamide (mg)	2.5 5		
Amiloride	5 10 15 20		

Page 1 of questionnaire

The following drugs were also listed in the questionnaire on subsequent pages:

Nifedipine retard, nicardipine, amlodipine, diltiazam, doxazosin, hydralazine, enalapril, captopril, lisinopril, perindopril, ramipril, fosinopril, trandolapril, cilazopril with a variety of dosages.

A3 Results

Four questionnaires were completed (80%), two by diabetologists and two by nephrologists. Only one of the four consultants completed the questionnaire on his own, the other three required detailed explanation from the author. From the questionnaires on preferred choice of anti-hypertensive therapy in Type 1 and Type 2 nephropathy patients, enalapril (an angiotensin converting enzyme inhibitor [ACEI]) was the preferred choice in both Type 1 and Type 2 patients with other ACEI coming about half way between enalapril and the least preferred treatment in both groups and other types of treatment varying in preference depending on the type of diabetes (Tables A3.1 and A3.2). Amlodipine was the calcium antagonists of choice used in both type of diabetes. Points were allocated for dosage of drugs as described in Table A3.3.

All four consultants added information on an algorithm for treatment with a consensus that ACEI are the first line of choice in patients with Type 1 diabetes and with caution in Type 2 patients, followed by either beta blockers, calcium channel blockers, alpha blockers or diuretics. One consultant favoured verapamil in Type 2 patients and two recommended doxazosin for uncontrolled systolic hypertension.

Drug	Average Mark	Grade	Points
ENALAPRIL	8.5	1	22
BISOPRILOL	7.0	2	21
ATENOLOL	6.8	3	20
DOXAZOSIN	6.5	4	19
LISINOPRIL	6.0	5	18
FRUSEMIDE	5.0	6	17
DITIAZEM	5.0	6	17
AMLODIPINE	5.0	6	17
BUMETAMIDE	4.3	9	14
NIFEDIPINE	4.0	10	13
PERINDOPRIL	4.0	10	13
RAMIPRIL	3.3	12	11
CAPTOPRIL	2.8	13	10
CELIPROLOL	2.5	14	9
VERAPAMIL	2.0	15	8
NICARDIPINE	1.8	16	7
CILAZOPRIL	1.8	16	7
TRANDOLAPRIL	1.8	16	7
FOSINOPRIL	1.8	16	7
INDAPAMIDE	1.3	20	3
HYDRALAZINE	1.3	20	3
AMILORIDE	0.5	22	1

Table A3.1: Points for different anti-hypertensive drugs in Type 1 nephropathy patients

Drug	Average Mark	Grade	Points
ENALAPRIL	6.50	1	22
DOXAZOSIN	5.25	2	21
AMLODIPINE	5.25	2	21
ATENOLOL	5.00	4	19
FRUSEMIDE	4.25	5	18
LISINOPRIL	3.75	6	17
BUMETAMIDE	3.25	7	16
DITIAZEM	3.25	7	16
NIFEDIPINE	2.75	9	14
BISOPROLOL	2.50	10	13
VERAPAMIL	2.00	11	12
PERINDOPRIL	2.00	11	12
CAPTOPRIL	1.50	13	10
RAMIPRIL	1.25	14	9
CELIPROLOL	0.75	15	8
INDAPAMIDE	0.50	16	7
HYDRALAZINE	0.50	16	7
NICARDIPINE	0.50	16	7
AMILORIDE	0.25	19	4
CILAZOPRIL	0.25	19	4
TRANDOLAPRIL	0.25	19	4
FOSINOPRIL	0.25	19	4

Table A3.2: Points for different anti-hypertensive drugs in Type 2 nephropathy patients

Drug	Dose	Points	Drug	Dose	Points
ENALAPRIL (mg)	2.50	2	ATENOLOL (mg)	25.00	4
	5.00	4		50.00	8
	10.00	6		100.00	8
	15.00	7			
	20.00	8	FRUSEMIDE (mg)	20.00	3
	30.00	10		40.00	5
	40.00	10		80.00	4
CAPTOPRIL (mg)				120.00	6
	12.50	2		160.00	6
	25.00	4			
	50.00	7	BISOPROLOL (mg)	5.00	5
	100.00	10		10.00	5
PERINDOPRIL (mg)	2.00	3	BUMETAMIDE (mg)	1.00	3
	4.00	5		2.00	6
	8.00	8		6.00	8
			DITIAZEM (mg)	60.00	2
LISINOPRIL (mg)	2.50	2		120.00	3
	5.00	3		180.00	5
	7.50	4		360.00	10
	10.00	5			
	15.00	6	CELIPROLOL (µg)	200.00	5
	20.00	7		300.00	7
	40.00	10		400.00	10
RAMIPRIL (mg)	1.25	3	INDAPAMIDE (mg)	2.50	2
	2.50	4		5.00	3
	5.00	7			
	10.00	10	HYDRALAZINE (mg)	25.00	2
				50.00	5
NIFEDIPINE RETARD (mg)	10.00	1		75.00	7
	20.00	3		100.00	9
	30.00	6		150.00	10
	40.00	8	NICARDIPINE (mg)		
	60.00	8		40.00	3
	90.00	10		60.00	5
				90.00	7
DOXAZOSIN (mg)	1.00	1		120.00	10
	2.00	3	AMILORIDE (mg)		
	4.00	4		5.00	1
	5.00	6		10.00	2
	8.00	9		15.00	0
				20.00	0
AMLODIPINE (mg)	5.00	5			
	7.50	7			
	10.00	10			

Figure A3.3: Points allocated for individual doses.

The mean points for anti-hypertensive treatment have been used in Chapter 8 to illustrate the differences between Type 1 and 2 patients. The following box plots of points in different ethnic groups show that this method has been useful in comparing anti-hypertensive treatment over several years, for identifying lack of treatment and for comparing different groups of patients (Figures A1-A3).

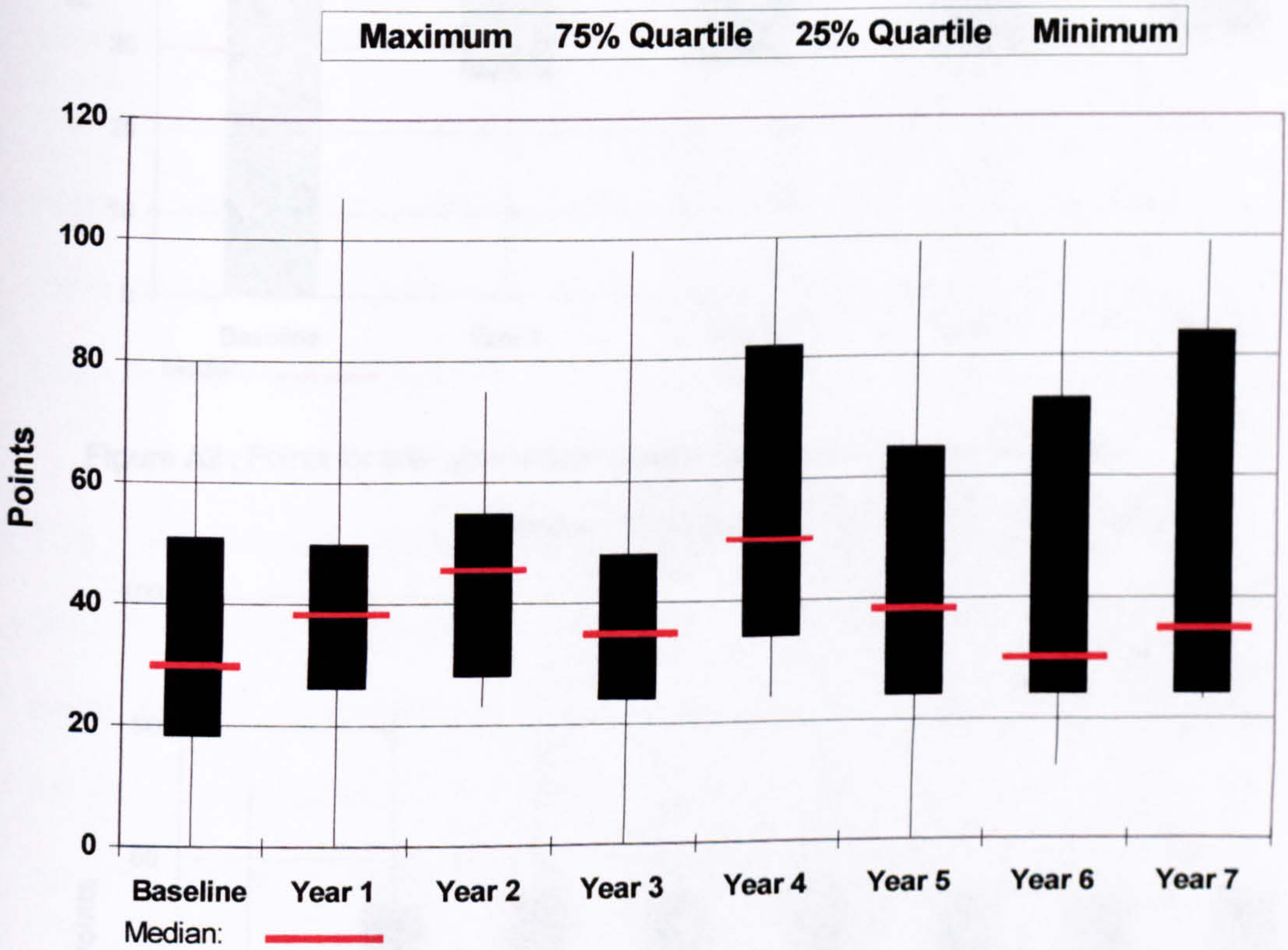

Figure A1 : Points for anti-hypertensive therapy in Indo-Asian patients over seven years

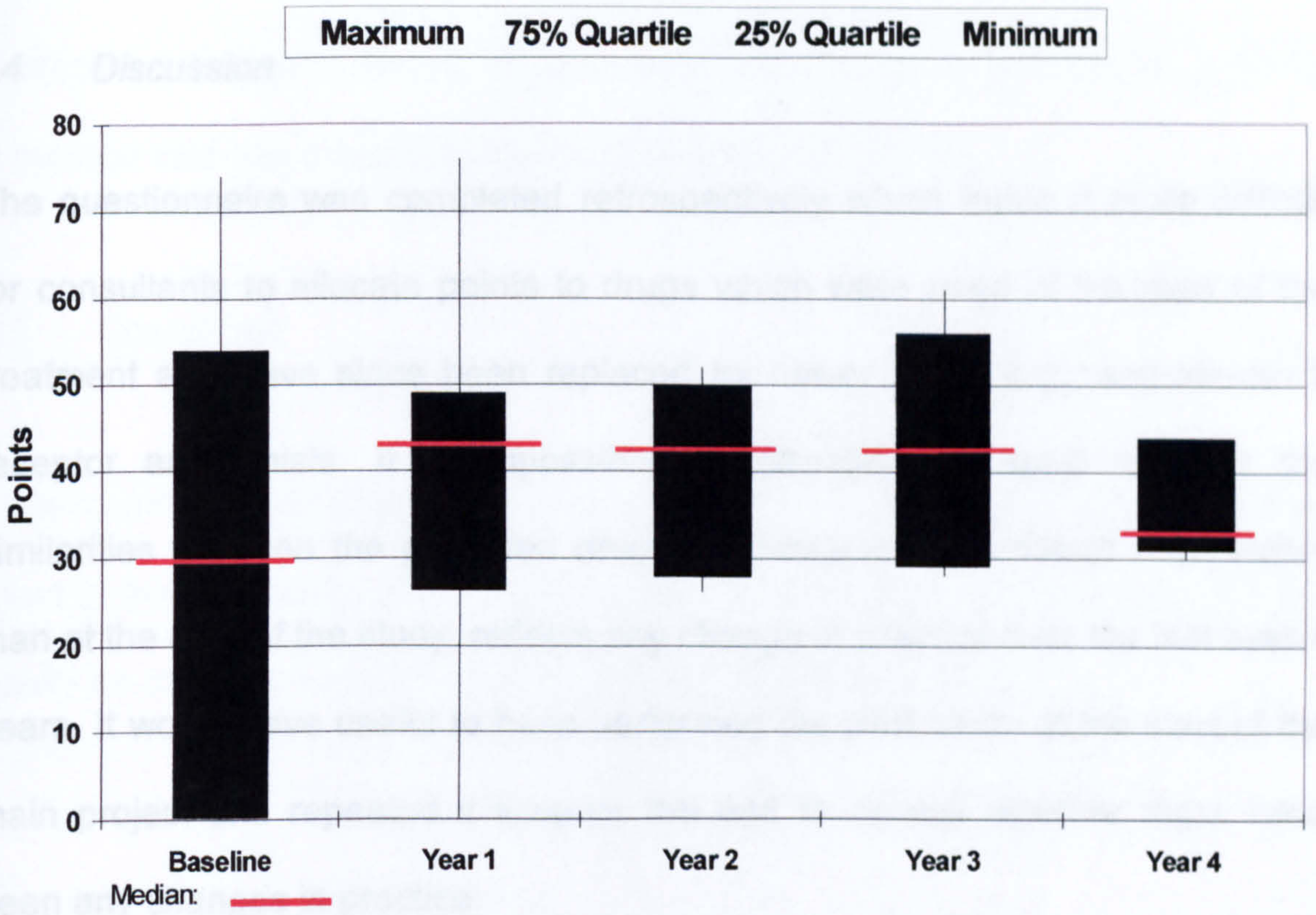

Figure A2 : Points for anti-hypertensive therapy in Black patients over four years

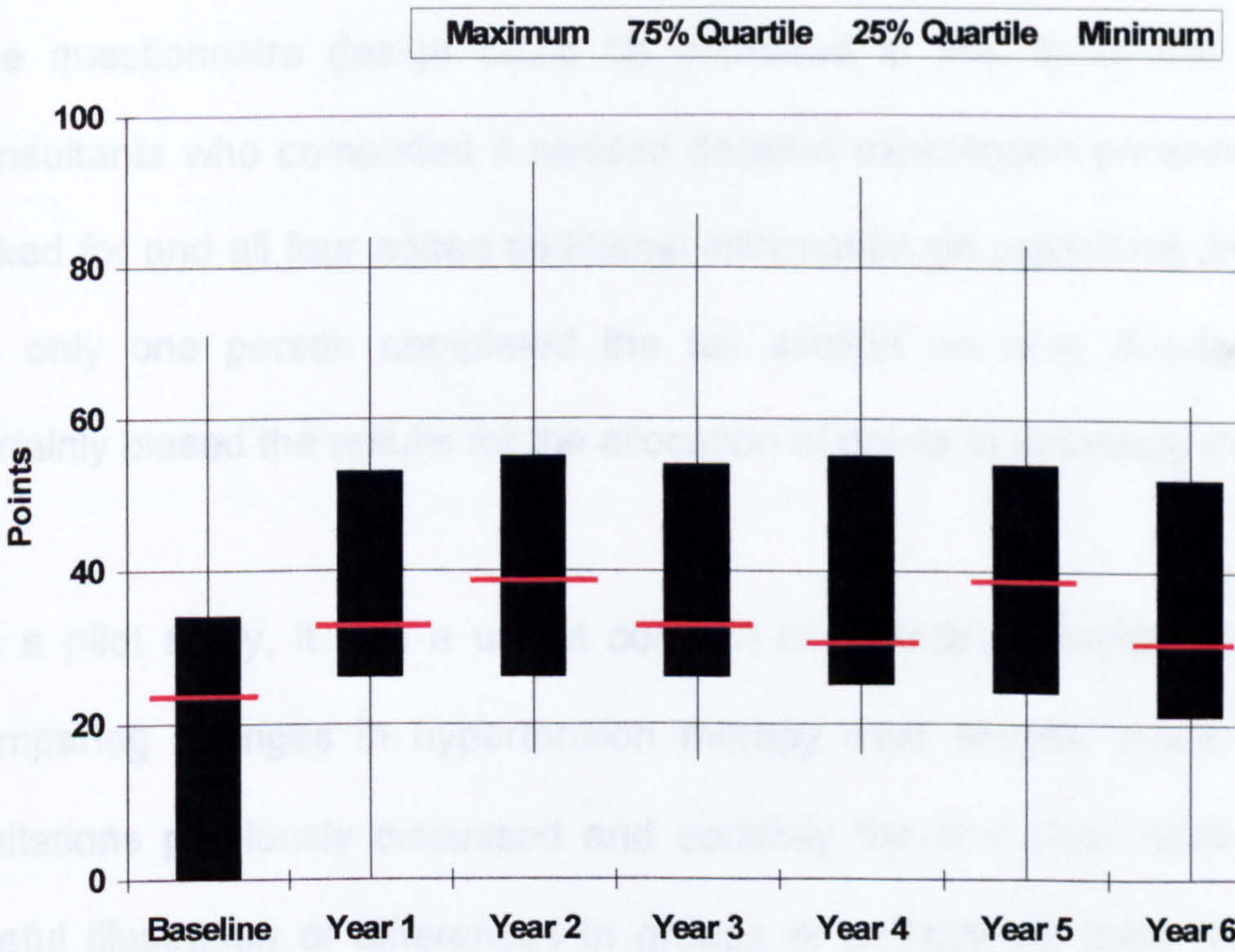

Figure A 3: Points for anti-hypertensive therapy in White patients over seven years

A4 Discussion

The questionnaire was completed retrospectively which made it more difficult for consultants to allocate points to drugs which were used at the start of the treatment and have since been replaced by newer drugs e.g.: angiotensin II receptor antagonists. It is impossible in retrospect to know whether the similarities between the preferred drugs of choice as determined now, rather than at the start of the study, reflects any change in practice over the last twelve years. It would have useful to have performed the pilot study at the start of the main project and repeated it towards the end to assess whether there have been any changes in practice.

The questionnaire design could be improved in that three out of the four consultants who completed it needed detailed explanation on what was being asked for and all four added additional information on algorithms for treatment. As only one person completed the full section on drug dosages this has certainly biased the results for the allocation of points to individual dosages.

As a pilot study, it was a useful concept to provide a quantitative means of comparing changes in hypertension therapy over several years despite the limitations previously discussed and certainly the box plots have provided a useful illustration of differences in groups of patients as have the graphs in Chapter 8 of mean point scales over different years.

Collecting information from a larger number of consultants would have provided a method that was a statistically sound quantitative tool.

A5 Conclusion

As a means of quantitatively determining anti-hypertensive therapy, the method used in this pilot study gives indications rather than definitive answers. It could easily be modified further for use in future studies if required.

Appendix 2

Definition of Terms

Alpha blockers: Alpha-adrenoceptor blocking drugs

Black : People of African origin irrespective of place of birth.

Beta blockers: Beta-adrenoceptor blocking drugs

Defaulting from routine clinic visits: Patients missed more than two consecutive clinic visits in more than one year since diagnosis.

Diabetic nephropathy: Presence of persistent proteinuria plus diabetic retinopathy (Clinical diagnosis) or confirmed by renal biopsy.

Hypertension: Systolic pressure $\geq$ 140 mm Hg and / or diastolic pressure $\geq$ 90 mm Hg or receiving anti-hypertensive therapy as defined by the Working Group on Hypertension in Diabetes, 1987.

Indo-Asian: People with origins from the Indian sub-continent i.e.: India, Pakistan and Bangladesh, irrespective of place of birth.

Ischaemic heart disease: Documented evidence of angina symptoms or electrocardiographic evidence.

Lost to follow up: Patient stopped attending nephrology and diabetic clinics plus no information available on the patient having died.

Peripheral vascular disease, severe: Presence of intermittent claudication or amputation.

Renal disease: Presence of persistent proteinuria on "stick" testing (confirmed by measurement of 24 hour urinary protein excretion) and / or raised serum creatinine concentrations (> 120 μmol l^{-1}).

Retinopathy, background: Presence of new blood vessels, cotton wool spots and dot or blot haemorrhages

Retinopathy, severe: Presence of proliferative changes, haemorrhage or loss of visual acuity not due to other conditions.

Type 1 diabetes: Diabetes mellitus diagnosed before age 35 years and requiring insulin treatment within the first year of diagnosis.

Type 2 diabetes: Diabetes mellitus diagnosed after 35 years of age.

White: People of European origin irrespective of place of birth.

Appendix 3

Specimen Death Certificate

From: *1995 Mortality statistics* (Office for National Statistics, 1997)

TEXT BOUND INTO

THE SPINE

Annex A Certificate used in the current system for certifying deaths

MED A 14 000000

197

BIRTHS AND DEATHS REGISTRATION ACT 1953

(Form prescribed by the Registration of Births, Deaths and Marriages (Amendment) Regulations 1985)

MEDICAL CERTIFICATE OF CAUSE OF DEATH

For use only by a Registered Medical Practitioner WHO HAS BEEN IN ATTENDANCE during the deceased's last illness, and to be delivered by him forthwith to the Registrar of Births and Deaths.

Registrar to enter No. of Death Entry

Name of deceased .....

Date of death as stated to me ..... day of ..... 19 ..... Age as stated to me .....

Place of death .....

Last seen alive by me ..... day of ..... 19 .....

1 The certified cause of death takes account of information obtained from post-mortem.
2 Information from post-mortem may be available later.
3 Post-mortem not being held.
4 I have reported this death to the Coroner for further action.

[See overleaf]

Please ring appropriate digit(s) and letter.

a Seen after death by me.
b Seen after death by another medical practitioner but not by me.
c Not seen after death by a medical practitioner.

CAUSE OF DEATH

The condition thought to be the 'Underlying Cause of Death' should appear in the lowest completed line of Part I.

	These particulars not to be entered in death register
I(a) Disease or condition directly leading to death†	Approximate interval between onset and death
(b) Other disease or condition, if any, leading to I(a)	
(c) Other disease or condition, if any, leading to I(b)	
II Other significant conditions CONTRIBUTING TO THE DEATH but not related to the disease or condition causing it.	

The death might have been due to or contributed to by the employment followed at some time by the deceased. ☐ Please tick where applicable

†This does not mean the mode of dying, such as heart failure, asphyxia, asthenia, etc: it means the disease, injury, or complication which caused death.

I hereby certify that I was in medical attendance during the above named deceased's last illness, and that the particulars and cause of death above written are true to the best of my knowledge and belief.

Signature ..... Qualifications as registered by General Medical Council .....

Residence ..... Date .....

For deaths in hospital: Please give the name of the consultant responsible for the [illegible]

Appendix 4

World Health Organization
International Classification of Disease
Ninth Edition

ICD-09 Code: 250: Diabetes Mellitus

246.2 *Cyst of thyroid*

Excludes: cystadenoma (226)

246.3 *Haemorrhage and infarction of thyroid*

246.8 *Other*

Abnormality of thyroid-binding globulin
Atrophy of thyroid

246.9 *Unspecified*

DISEASES OF OTHER ENDOCRINE GLANDS (250-259)

250 Diabetes mellitus

The following fifth-digit subclassification may be used, if desired, with category 250:

.0 adult-onset type
.1 juvenile type
.9 unspecified whether adult-onset or juvenile type

Clinical information should be obtained when choosing the fifth digit. If impossible, .9 should be used.

Excludes: neonatal diabetes mellitus (775.1)
nonclinical diabetes (790.2)
when complicating pregnancy, childbirth or the puerperium (648.0)

250.0 *Diabetes mellitus without mention of complication*

Diabetes mellitus without mention of complication or manifestation classifiable to 250.1-250.9
Diabetes (mellitus) NOS
~~Diabetic arteriosclerosis~~ unless ketoacidosis or coma is mentioned, ~~with 440.9~~
~~Diabetic ulcer~~

250.1 *Diabetes with ketoacidosis*

Diabetic: acidosis, ketosis } without mention of coma

250.2 *Diabetes with coma*

Diabetic coma (with ketoacidosis)
Diabetes with hyperosmolar coma

250.3† *Diabetes with renal manifestations* (581.8, 582.8, 583.8*)

Diabetic nephropathy
Intracapillary glomerulosclerosis
Kimmelstiel-Wilson syndrome

250.4† *Diabetes with ophthalmic manifestations*

Diabetic:
cataract (366.4*)
retinopathy (362.0*)

250.5† *Diabetes with neurological manifestations*
Diabetic:
amyotrophy (358.1*) Diabetic polyneuropathy (357.2*)
mononeuropathy (354.–, 355.–*)

250.6† *Diabetes with peripheral circulatory disorders*
Diabetic:
gangrene (785.4*)
peripheral angiopathy (443.8*)

250.7† *Diabetes with other specified manifestations*
Excludes: intercurrent infections in diabetic patients

250.9 *Diabetes with unspecified complications*

251 Other disorders of pancreatic internal secretion

251.0 *Hypoglycaemic coma*
Iatrogenic hyperinsulinism Insulin coma
Use additional E code, if desired, to identify cause if drug induced

251.1 *Other hyperinsulinism*
Hyperinsulinism:
NOS
ectopic
functional
Hyperplasia of pancreatic islet beta cells NOS

251.2 *Hypoglycaemia, unspecified*

251.3 *Postsurgical hypoinsulinaemia*
Postpancreatectomy hyperglycaemia

251.4 *Abnormality of secretion of glucagon*
Hyperplasia of pancreatic islet alpha cells with glucagon excess

251.5 *Abnormality of secretion of gastrin*
Hyperplasia of pancreatic alpha cells with gastrin excess
Zollinger-Ellison syndrome

251.8 *Other*

251.9 *Unspecified*
Islet-cell hyperplasia NOS

252 Disorders of parathyroid gland

252.0 *Hyperparathyroidism*
Hyperplasia of parathyroid
Osteitis fibrosa cystica generalisata [von Recklinghausen's disease of bone]
Excludes: secondary hyperparathyroidism (of renal origin) (588.8)

Appendix 5

Standard Operating Procedures for Biochemical Tests

INTRODUCTION

The Biomen 8140 Analyser measures HbA1c by High Pressure Liquid Chromatography. When operated in the batch mode, the analyser will produce a result in about four minutes. The use of the prehaemolysed feature enables the department to offer up to the minute results on patients attending the paediatric diabetic clinic.

HbA1c is well established as an indicator of diabetic control over a period of about 3 months, the average life span of a red blood cell being about 100 days. The system does not detect abnormal haemoglobins, thus patients with these variants can be difficult to recognise and they are better monitored by fructosamine estimation.

MAINTENANCE AND SIMPLE DIAGNOSTICS

The 8140 has proven to be a very reliable analyser. It is vital that the maintenance as described on the maintenance sheet is absolutely adhered to. This will keep problems to a minimum. Turning the column regularly will approximately double the life of the column to about 5000 tests. Filters may need changing more often than every 500 samples as they are prone to blockage. It is crucial to empty the waste container after each run: if the waste reaches a certain level, the analyser will automatically shut down. Failure to close the manifold screw will cause the waste bottle to fill rapidly and again shutdown will occur. The usual operating pressure is 57. Changing the filter or turning the column will not affect this by more than 2 if it has been done correctly. When working on the system ***never*** use excessive force, as it is possible to shear the precision fittings. Frequently the frits that form seals become deformed and can be difficult to remove. The solution is to carefully prise them out with thin scissors, thin scalpel blades etc. The 8140 is supplied with a comprehensive handbook particular has. If a problem proves difficult, the technical backup provided by the company is immaculate.

REAGENTS

All of the reagents are supplied by Biomen:-
Eluant 21A
Eluant 21B
Eluant C
Haemolysant.

CONTROLS

2 level 'Glyco HB Control' code U 8312 supplied by Menari Diagnostics.

BATCH MODE OPERATION

Introduction

A worksheet is automatically generated when 100 unknowns have been reached. If desired, a wok list can be forced to be printed using 'EDT' and 'worksheet'. The analysis of a batch has been considerably simplified by the use of a PC to link generation of information from the 8121 to the LDM. The stages involve downloading a worksheet, creating a load list, performing the batch run and uploading the data to LDM.

Method

On the 'speedmaster' click on the flame icon. The LMX/HbA1 Analyser Interface will be displayed. Click on 'Get Worksheet'. A box will be displayed asking for the number of the worksheet to be downloaded. Enter the number and click OK. An acknowledgement will appear in the Biomen window and a few seconds later the worksheet will be scrolled upwards in the LDM window. When downloading is complete, it is necessary to close the application before attempting to create the load list. Re-open the application by clicking on the flame icon. In a few seconds the Analyser Interface will be displayed. Create the load list by clicking on 'Create Load List'. You will be asked for the total number of samples you wish to analyse. Enter the number in the run plus one to allow for the batch check. Click on create, wait for five seconds then click on done and close. The load list will be printed.

Load the samples on according to the load list, checking each sample carefully. The probe is cap piercing. ***Mistakes at this stage can be impossible to unravel.*** Press start and click on 'receive data'. If you forget this last step and do it when some results have gone across, the data comes across in an avalanche and is usually garbled; do not forget!

ANALYSIS OF CONTROLS AND CLINIC SAMPLES

Controls and clinic samples are run pre-haemolysed. The haemolysate is prepared by adding 20ul of whole blood to 2.7mls of haemolysing solution an a sugar tube mixing to ensure an even haemolysant. The 8140 will recognise samples as pre haemolysed if the first four tube wells in a sample rack are lect empty. All six remaining sample wells will be treated as prehaemolysed. The results will be printed with a '999' preceeding the result, thus prehaemolysedsample number one will be printed as '9991', sample two as '9992' etc. Before uploading the results to LMX it is necessary to remove the pre-haemolysed results.

Removal of Pre-haemolysed samples

Click on Files.
Click on Edit Biomen Data.
Find 9991 and 9992 lines by scrolling, highlight them and press delete twice to delete them and remove an unwanted carriage return.
Click on Files.
Click on Save Biomen Data.
Click on Files.
Click on Regenerate Results.

At the end of the run, click on 'stop analyser link, and then click on 'upload to LMX'. The process of uploading is relatively slow and it is prudent to allow half an hour for this process, if LDM is busy. The data are sent to individual patient files by using EFT, ERFT and the relevant work list number. The results will scroll rapidly up the page. If any analyses have not

produced results, a space will occur and you may enter a result manually or send the analysis to another worksheet using 'replig'.

HEALTH and SAFETY
Refer to COSSH Assessment Ref. No. COA18

INTRODUCTION

The RA 1000 is basically a combined pipetting station and spectrophotometer with temperature control. It is both accurate and precise, typical cv's being < 3%. It performs a wide range of tests, the mode of operation is similar for each. Consequently this procedure consists of some specific details about the individual methods and a flow chart explaining how to run the instrument, with details of how to calibrate and simple troubleshooting.

The methods fall into two groups....

1 Methods using manufacturers kits.
2 Methods using reagents prepared in the laboratory.

The parameters for each test are stored in the RA, the parameter list contains the settings that are stored in the RA 1000 to enable analyses to be performed. Each set of parameters is unique. Parameters can be listed using 94 function and the RA chemistry number. One example is given below.

Glucose Parameters Chemistry 13

NAMEGLUC
TYPE 1
% SMP VOL 6
FILTER POS 4 WL 500
DELAY 0 30
INCUBATION 1 00
% RGT VOL 74
UNITS4 MMOL/L
UNIT FAC 1.0000
DECIMAL PT1
RBL LOW 0.000
RBL HI 0.200
RANGE LOW 0
RANGE HI 25
CAL FACTOR 188.68
RGT RATE 0.0000
STD VAL 16.3
NORMAL LO 2.0
NORMAL HI 8.0
SLOPE 1.000
INTERCEPT 0.00
LIN FACT 1.00
1ST LIM 1.000

REFERENCE

Technicon RA1000 handbooks.

The Royal Wolverhampton Hospitals NHS Trust
Department of Clinical Chemistry

STANDARD OPERATING PROCEDURE FOR THE RA 1000

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REFERENCES

The wide range of tests offered by the RA 1000 have extensive clinical utility, which is described in several textbooks. 'Clinical Chemistry' by Marshall has excellent clinical information, whilst 'Clinical Chemistry in Diagnosis and Treatment' by Mayne is more biochemically based.

QUALITY CONTROL

Bio-rad 2 level control supplied by Bio-rad Laboratories, Maylands Avenue, Hemel Hempstead, HP2 7TD. Add 10ml of deionised water to each level and mix for 30 minutes. Stable for 3 days at 4°C

HDL CHOLESTEROL & TOTAL CHOLESTEROL

1.5g glycine
Make up to 100ml with water. Store

- Reagents supplied by Bayer Diagnostics Product number T01 1684 01
- Standard : Randox Level I, diluted 1/2 with distilled water. The dilution does not affect the results , as the samples are diluted by the same factor during pre-treatment.
- QC material : HIQC 17.
- Working reagent stability : One month at 4°C
- Calibration frequency : Calibrate before each run.
- Normal range : Male 0.7 - 1.9 mmol/l Female 0.7 - 2.0 mmol/l

Add 200ul PEG, vortex mix.

- Analyse the supernatant for cholesterol.

Cholesterol esterase hydrolyses cholesterol esters to free cholesterol, which in the presence of oxygen and cholesterol oxidase produces hydrogen peroxide, which in turn produces a quinoneimine dye. A red colour is produced which is measured at 525nm

$$\text{Cholesterol esters} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol and fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest - 4 en - 3 - one} + H_2O_2$$

$$H_2O_2 + \text{4 - Aminoantipyrene} + \text{p-Hydroxybenzoate} \xrightarrow{\text{Peroxidase}} 4H_2O + CO_2 + \text{Quinoneimine dye}$$

CREATININE

N. B. Where the following symbol ☠ occurs next to a reagent, look up the hazards and precautions to be taken in the BDH Hazard Data Sheets.

- Reagents supplied by Bayer Diagnostics.
- Picric Acid. Product number T21-1928-A1 ☠ (EXPLOSIVE WHEN DRY).
- NaOH. Product number T21-1929-A1.
- Working reagent. Mix equal volumes of Picric acid reagent and NaOH reagent Add 1 drop of wetting agent for every 7ml of reagent. Stable for 1 month at room temperature.
- Standard Randox Level II
- QC Material Nycomed three level controls.
- Calibration frequency As judged by control values
- Normal range 60 - 120 umol/l.

Creatinine reacts with picric acid in an alkaline medium to produce a red coloured complex, in a selected time interval, is proportional to the original creatinine concentration. The absorbance of the analytical mixture is measured at 500 nm on a first order reaction curve. The reaction is initiated by addition of sample to the reagent. Interference may result from extremely jaundiced samples.

PLASMA LACTATE

- RA chemistry number 32
- Tray 4: Position 1.
- Normal Range 1.0 - 1.8 mmol/l
- QC material : Batch check from previous analysis.
- Standard : Lactate Standard (Sigma 51h 6151) . A working standard, 4.44 mmol/l is prepared by diluting this stock standard 1/3 with 0.6 mmol/l $HClO_4$.
- Calibration frequency : Standardisation is necessary each time.

Reagents

N. B. Where the following symbol ☠ occurs next to a reagent, look up the hazards and precautions to be taken in the BDH Hazard Data Sheets.

- **1 0.6M HCl O_4** ☠ (EXPLOSIVE WHEN DRY) 64.5 ml 60-62% perchloric acid (BDH) diluted to 1 litre with distilled water.
- **2 Lactate Buffer, pH 9.15**. 40g glycine ☠ (BDH), 20ml AnalaR Hydrazine Hydrate ☠ (BDH), 4g EDTA, disodium salt ☠ (BDH). Adjust to pH 9.15 with 10% LiOH ☠ (BDH) and make up to 1 litre with distilled water.
- **3 B - NAD** ☠ (Sigma 12H7843). Store at less than 0°C.
- **4 LDH solution** ☠ (Sigma 10H96150). Store at 4°C.

Working lactate reagent and stability

Make up as follows in a 25ml universal container:

- 55mg NAD
- 10 ul LDH solution
- 25 ml Lactate buffer

This reagent will last if stored at 4°C for one week. However the LDH needs topping up each time, as it will gradually lose activity. Add 1ul each time the reagent is used.

Method

Plasma from a fluoride oxalate tube is required. Lactate is stable in this tube for at least 4 hr. The sample must be prepared for analysis immediately upon receipt. Centrifuge the sample for 5 min at 3000 rpm. Make a 1/3 dilution of plasma in cold 0.6M $HClO_4$ using 0.2 ml plasma and 0.4ml acid in a plastic bullet tube. Vortex and allow to stand for 5 minutes. Centrifuge in the micro centrifuge for 1 minute at 13,000rpm. Collect the supernatant into a new bullet tube. The lactate is now stable.

LDH catalyses the reversible conversion of lactate to pyruvate;

$$\text{L - Lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{pyruvate} + \text{NADH}$$

The reaction is driven to the right by using excess NAD and hydrazine which removes pyruvate as soon as it is formed.

URINE PROTEIN

RA chemistry number 33 Work station number 2.
Tray 4: Position 6.
Normal range: Up to 100mg/24 hr. NB values >2.0 g/l require dilution.

Reagents

N. B. Where the following symbol ☠ occurs next to a reagent, look up the hazards and precautions to be taken in the BDH Hazard Data Sheets.

- **Pyrogallol red** ☠ 1.5mmol/l . Dissolve 60mg Pyrogallol red (Sigma product 8759) in 100ml methanol BDH (GPR).
- **Sodium Molybdate.** Dissolve 0.24g disodium molybdate.$2H_2O$ ☠ in 10ml distilled water.
- **Buffer.** Dissolve 5.9g succinic acid ☠ (BDH), 0.14g sodium oxalate ☠ (BDH) and 0.5g sodium benzoate ☠ (BDH) in 900ml distilled water.
- **Working reagent** To the 900ml of buffer, add 40ml pyrogallol solution, 4ml of the sodium molybdate solution. Adjust to pH 2.50 with 0.1M HCl and dilute to 1 litre with distilled water. Add 1ml/l of 30% Brij solution. Stable for 6 months at 4°C.

Standard

Aqueous human albumin (BDH) concentration 1.60g/l.

QC Material

Aqueous human albumin (BDH) concentration 1.60g/l.

Calibration frequency

As judged on the basis of control values.

Under acidic conditions and in the presence of molybdate ions, proteins form a blue coloured complex with pyrogallol red. The absorbance is measured at 600nm.

OPERATION OF THE RA 1000

Check that the daily maintenance has been performed. Check that there is sufficient reagent for the number of tests that you wish to perform. Ensure that you have installed the correct reagent tray. These are stored in the RA's memory and for convenience are also displayed on a card between the machines. The RA is a 'random access' analyser enabling any combination of tests on a selected reagent tray to be performed. Results are printed on the paper roll and may also be sent directly to the main computer.

Periodic calibration of certain analyses may be required. Calibrating the RA is described subsequently as a sub routine of running a work list. Individual analyses are performed in separate cuvettes of the plastic rotor tray. There are 100 cuvettes per tray. When the last cuvette has been filled the alarm will sound and the LED will display 'RT FULL'.

CHANGING THE TRAY

Press 'clear entry' to switch the alarm off. Remove the tray and empty the contents into VIRKON tank 1, then immerse the tray in VIRKON tank 2, CARE!! Introduce a clean tray and press '30 function'. This will command the RA to scan the cuvettes. The number of unsatisfactory cuvettes will be displayed on the LED e.g. '2 bad cups', these will not be used for analyses. Press '0 operate enter'; the RA will perform the rest of the tests.

ROTOR TRAY WASHING WITH VIRKON

Virkon Medical Disinfectant is obtained from the hospital pharmacy. The 5kg tin is stored in the fume cupboard in the weighing room. **N.B. Always wear gloves and glasses when working with virkon.**

Every Monday, empty the two virkon tanks. Tank 1 contains 180g of virkon in 4 litres of water. This tank receives the contents of the used tray. tank 2 contains 360g of virkon in 8 litres of water in which the rotors are soaked for at least 8 hours to disinfect. A measuring cup is provided with the virkon. It is important to void the rotors completely into tank 1, to minimise precipitation in tank 2.

Tank 2 should be emptied each day. To minimise time - consuming washing at weekends, it is prudent to build up a stock of ' W11' trays. Trays that have been used twice should be discarded. Rinse trays thoroughly in tap water, then place the tray in the Biostat washer and wash twice with distilled water. Do not allow trays to dry out before the distilled water wash.

After washing, dry the trays on the oven in the wash - up room. When dry, inspect each cuvette, polishing if necessary with a clean cloth. Dirty cups should be blacked out so that they will not be used. Mark the number of washes on the tray, e.g. 'W1' or 'W11'. Place the tray in a plastic bag and store in the cardboard box provided.

The Royal Wolverhampton Hospitals NHS Trust
Department of Clinical Chemistry

HbA1c (HbA1)

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PRINCIPLE

The Technicon DAX multichannel analyser has the capability to perform up to 24 tests on a sample, e.g. Sodium, potassium, bicarbonate, urea, creatinine, albumin, total protein, bilirubin, alkaline phosphatase, alanine transaminase, lactate dehydrogenase, magnesium, urate, calcium, phosphate, cholesterol and triglycerides. Sodium and potassium are measured using ion - selective electrodes. Other analyses depend on changes in ultra - violet and visible light.

REFERENCES

The wide range of tests offered by the DAX have extensive clinical utility, which is described in several textbooks. 'Clinical Chemistry' by Marshall has excellent clinical information, whilst
'Clinical Chemistry in Diagnosis and Treatment' by Mayne is more biochemically based. Specific information about the methods is available in the DAX methods manual.

REAGENTS SUPPLIED BY BAYER DIAGNOSTICS

Dax Reagent	Part Number	Reagent Stability
Albumin	011377B3	Expiry date as per container, stored at R.T.
Alk Phos (DEA)	011902A0	Expiry as per bottle, 7 days reconstituted 4-8 deg
ALT	011760A0	Expiry as per bottle, 7 days reconstituted 4-8 deg
AlT Reagent 2	111762C0	Ditto
AST	011750B5	Ditto
AST Reagent 2	111752C0	Ditto
Creatinine		Individual components expiry as container, 10 days at R.T. once mixed
Calcium	011476A0	Expiry date as per container, stored at 4-8 deg
Calcium Reagent 2	211516C0	Ditto
Cholesterol	011684A0	Expiry as per container, 10 days at 4-8 deg reconstituted
P5P	11336262	
Total Bilirubin	011963A0	
Total Protein	011301B3	Expiry as per container, store at R.T.
Triglyceride	011863B5	
Urea	011823A0	
Uric Acid	012577B5	
ISE Acid Wash	013164A1	Expiry as per container, store at R.T.
ISE Alkaline Wash	013165A1	Ditto
Bath Additive	013163A1	Ditto
ISE Buffer	013161A1	Ditto
ISE Detergent	01358554	Ditto
Mid - Control Calib	033162A1	Ditto

The Royal Wolverhampton Hospitals NHS Trust
Department of Clinical Chemistry

STANDARD OPERATING PROCEDURE FOR THE DAX 24

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CALIBRATORS

Three calibrators are used:-

1□ ISE standards as supplied by Bayer Diagnostics. Part numbers-Low 03-3166-A0, High 03-3067-A0.

2□ DAX calibrator supplied by Bayer Diagnostics. Part number 03321762.

3□ In house bicarbonate standard (25 mmol/l)

QUALITY CONTROL

Biorad two level control. Details of analytical performance can be found in the DAX methods manual.

METHOD

Start Up and Reagent Preparation

- Most of the maintenance and start up can be done setting the DAX to come on automatically at 8am.The instrument will be ready for use about 45 minutes later.
- Log on touch screen.
- Ensure that both green lights at the back of the water supply unit are on.
- Reagent status is checked at the end of the previous days work, as some reagents need special preparation. Add reagents as required. For enzyme tests, dissolve given quantities of powder with the diluent provided. These reagents can be used as soon as they have dissolved.
- Non refrigerated liquid reagents are ready for immediate use.
- Triglyceride reagent is prepared 24 hours before use.
- Urea is prepared 24 hrs before use.
- ALT reagents are made up in special bottles, as those supplied are too small to hold the required volume.
- Working bilirubin reagent is prepared by mixing reagents 1 and 2. The bilirubin blank is ready for use.
- Activate UIW screen.

Reagent Base Lines, Calibration and Loading of Controls

- Prime as necessary.
- Load a blue rack with one empty z10 tube for RBL.
- Load a green rack with DAX calibrant in position 1, ISE low calibrant in position 2 and ISE high calibrant in position 3. Single channels can be calibrated via menu → result validation → run condition.
- Load a yellow rack with controls and one z10 containing water for creatinine blank in position one.
- To commence RBL and calibration, press start/stop, run, yes.
- **N.B.** Dax does **not** do an automatic RBL when calibrating. Individual RBLs can be performed by highlighting those desired.

Analysing samples

- Load the control rack (yellow) at the beginning, followed by sample racks (white).
- Ensure the spout part of the insert is directly behind the bar code.
- Ensure that the bar codes face the reader.
- To start a run, press START/STOP, then RUN.

RESULTS

Calculation Include all calculations even if LMX does them for you.

Dilutions

The autodilution levels are pre-set by the manufacturers. There are four levels; Neat, 1:2, 1:4, 1:8

Action Limits

A list of action limits is kept alongside the analyser, and results limits are set in the UIW so that results outside the limits appear in the incomplete file for the operator to view them.

LIMITATIONS

The following analytes are affected by haemolysis and should not be reported on haemolysed samples (or samples which have been left on cells for excessive periods of time:-
Sodium, Potassium, Bicarbonate, ALT, Alk Phos.
Analytes on lipaemic samples should not be reported except for triglyceride and cholesterol. If electrolytes are required on suh samples, they should be analysed on the direct reading ISEs of the Opera.
Very jaundiced samples i.e. specimens with bilirubin greater than 100 umol/l should not have their creatinine reported.

HEALTH and SAFETY

Refer to COSSH Assessment Ref. No. COA11, CO24 and COA25

Authors Month Year

Appendix 6

Publications From This Research

EUROPEAN RENAL ASSOCIATION

EUROPEAN DIALYSIS AND TRANSPLANT ASSOCIATION

ABSTRACTS

XXXIV Congress of the
European Renal Association
European Dialysis and Transplant Association
September 21- 24, 1997
Geneva, Switzerland

RISK FACTORS FOR DEVELOPING NEPHROPATHY AND COMPLICATIONS IN PATIENTS WITH TYPE II DIABETES

EV McLelland, PB Rylance, J Odum, MA Jackson, S Walford, BM Singh, JD Phillips, C Padgham.
Renal & Diabetic Units, New Cross Hospital, Wolverhampton & Division of Clinical Science, University of Wolverhampton, U.K

Prevention of diabetic nephropathy and complications requires control of risk factors. A study was therefore performed to identify the risk factors in patients with Type II diabetes.
85 Type II patients with diabetic nephropathy were compared, at referral for nephrological assessment, with 76 Type II control patients without nephropathy, controlled for age (62 ±8 years), sex (68% male, 32% female), duration of diabetes (13 ±7 years) and ethnic group (Caucasian, Asian, Afro-Caribbean).
Type II nephropaths, when compared with controls, had increased retinopathy (95% v 54%, $p<0.001$), reduced visual acuity (25% v 9%, $p<0.001$), peripheral neuropathy (46% v 28%, $p<0.02$) and leg ulcers (20% v 1%, $p<0.001$). Nephropathy patients were more commonly hypertensive (≥140/90) (96% v 58%, $p<0.001$), and blood pressure was higher (mean systolic 173mmHg v 150, $p<0.0001$) diastolic 90 v 81, $p<0.005$). However, only 45% of nephropaths were on antihypertensives, compared with 91% controls ($p<0.001$). In addition more nephropaths had defaulted from clinic visits (44% v 8%, $p<0.001$). Diabetic control over a 9 year period was poor (HbA1c 10.2-12.2%) but there was no difference between the two groups. More nephropaths had previously smoked (51% v 24%, $p<0.001$), and still smoked (27% v 12%, $p<0.02$).
Type II diabetics with nephropathy have increased diabetic complications. Whilst diabetic control does not appear to be a risk factor, uncontrolled hypertension, failure to prescribe or take antihypertensives, defaulting from clinic and smoking are associated with the development of diabetic nephropathy in Type II patients. Clinical management of Type II diabetics should address these risk factors, in order to reduce development of nephropathy and other diabetic complications

INCREASED PREVALENCE AND ACCELERATED ONSET OF RENAL DISEASE IN PATIENTS WITH TYPE II DIABETES

EV McLelland, PB Rylance, J Odum, MA Jackson, S Walford, BM Singh, JD Phillips, C Padgham.
Renal & Diabetic Units, New Cross Hospital, Wolverhampton & Division of Clinical Science, University of Wolverhampton, U.K

There has been a progressive increase in referral of older diabetic patients with end-stage renal disease (ESRF), associated with increased morbidity. This study has assessed and prospectively followed 220 diabetics (7% of diabetic clinic patients) referred with proteinuria &/or raised creatinine over an 8 year period. Type I patients were compared with Type II.

78% of diabetics with renal disease were Type II, and were predominantly male (61%). 49% of Type II had become insulin-dependant. Development of renal disease was accelerated in Type II (11±8 years), compared with Type I (23±10, $p<0.001$). Type II were 20 years older than Type I (63±8 v 43±12 years, $p<0.001$). 96% Type I had diabetic nephropathy but only 60% Type II had diabetic nephropathy, the remainder had predominantly hypertension and reno-vascular disease, which correlated with the 41% with no diabetic retinopathy. There was a high incidence of macrovascular complications, particularly Ischaemic Heart Disease (16% v 19%, NS) and Peripheral Vascular Disease (39% v 29%, NS). Hypertension (≥140/90) was present in 90% Type I (mean 160/90) and 92% Type II (168/90), but only 46% Type I and 48% Type II were on antihypertensive therapy at referral. Diabetic control was poor in both groups (HbA1c 11.1% v 10.9%, NS), and cholesterol was similar (6.8 v 6.6mmol/l, NS). 26 Type II developed ESRF compared with 16 Type I. 40 died (18%), 17 (43%) from cardiovascular disease.

There is an increased prevalence of Type II diabetics with renal disease compared with Type I. Uncontrolled hypertension may be a factor in development of renal disease in both groups. In Type II patients age and renovascular disease may result in accelerated development of renal disease.